# EXPERIENTIA

EST EXPERIENTIA SCIENTIA PARACELSUS

REVUE MENSUELLE DES SCIENCES PURES ET APPLIQUÉES
MONATSSCHRIFT FÜR DAS GESAMTE GEBIET DER NATURWISSENSCHAFT
RIVISTA MENSILE DI SCIENZE PURE E APPLICATE
MONTHLY JOURNAL OF PURE AND APPLIED SCIENCE



UNIVERSITY OF HAWAII LIBRARY
FEB 20 '54



Editores:

R. MATTHEY (Lausanne) · A. v. MURALT (Bern) · L. RUZICKA (Zürich) · J. WEIGLE (Genève-Pasadena)

Redactor: H. MISLIN, Basel



VERLAG BIRKHÄUSER AG. · BASEL 10

SUISSE — SCHWEIZ — SVIZZERA — SWITZERLAND


SOMMAIRE - INHALT - SOMMARIO - CONTENTS


L. ZECHMEISTER: Some Stereochemical Aspects of Polyenes . . . . . 1

*Brèves communications - Kurze Mitteilungen - Brevi comunicazioni - Brief Reports*

V. HAHN, P. HAMMES und Z. GERIĆ: Die Konstitution des Phthalylharnstoffs . . . . . 11
W. BENCZE und H. SCHMID: Über die Konstitution des Visamminols . . . . . 12
C. W. BECK: The Effect of Hyperconjugation upon the Boiling Points of Alkenes . . . . . 14
H. LÜCK: Zur bakteriziden Wirkung von komprimiertem Sauerstoff . . . . . 15
G. CAVALLO: Inattivazione del profago a mezzo del calore . . . . . 16
J. PACLT: On the Pigment of *Chaetomium* sp., the Cause of Green Stain of Heart-wood in *Fagus sylvatica* . . . . 17
R. MATTHEY: Un nouveau type de chromosomes sexuels chez un mammifères . . . . . 18
MARY ESTHER GAULDEN: Telophase Behavior of Extranuclear Chromatin and its Bearing on Telophase Changes in Chromosomes . . . . . 18
(Mme) A. FICQ: Analyse de l'induction neurale par autoradiographie . . . . . 20
R. REITLER: Susceptibility to *Haemophilus Influenzae* Infection in Relation to the Age Factor . . . . . 22
G. HEIN: Der Rucktanz als wesentlicher Bestandteil der Bienentänze . . . . . 23
P. DE NICOLA and P. ROSTI: Method for the Quantitative Evaluation of Platelet Function in Blood Coagulation 24
E. SALVIDIO and E. URBANI: The Influence of the Nucleus and of Heat Regulation on the Dipeptidase Activity of the Nucleated and Anucleated Erythrocytes of Vertebrates . . . . . 25
G. DICASTRO: Some Remarks on Two-Dimensional Paper Electrophoresis . . . . . 27
A. FONNESU and CLARA SEVERI: Phosphorylation Mechanisms in Cloudy Swelling . . . . . 28
H. VAN CAUWENBERGE, J. LECOMTE et J. GOBLET: Essais d'inhibition de l'action inflammatoire cutanée du chloroforme chez le rat . . . . . 30
J. JÄNNES: Interrelationship between Vitamin $B_{12}$ and Pantothenic Acid in the Metabolism of "Wild" Strains of *Escherichia coli* . . . . . 31
J. JACOB: Sur l'inhibition sélective des acétylcholinestérases *in vivo* chez le chien . . . . . 33
E. HÄBERLI: Eine Methode zur titrimetrischen Bestimmung des Eisens im Blut mit Hilfe von Komplexon (Pro Experimentis) . . . . . 34

*Nouveaux livres - Buchbesprechungen - Recensioni - Reviews*

Methoden und Anwendungen der Massenspektroskopie. Von H. Ewald und H. Hintenberger (Verlag Chemie, Weinheim 1952) (Ref. M. J. Higatsberger) . . . . . 35
Chemistry of Carbon Compounds. Edited by E. H. Rodd, Vol. I B. 1952 and Vol. II A. 1953 (Elsevier Publishing Co., Amsterdam) (Ref. H. Dahn) . . . . . 36
Marine Geology. By Ph. H. Kuenen (John Wiley & Sons, Inc., New York, 1950) (Ref. M. Pfannenstiel) . . . . . 36
Modern Trends in Physiology and Biochemistry. Edited by E. S. Guzman Barron (Academic Press Inc. Publ., New York, 1952) (Ref. H. Nüesch) . . . . . 37

*Informations - Informationen - Informazioni - Notes*

Studiorum progressus: CH. GRÉGOIRE, GHISLAINE DUCHATEAU et M. FLORKIN: La trame protidique des nacres . . 37

# EXPERIENTIA

L'Experientia publiera:

1. des *articles originaux* sur les récentes recherches scientifiques écrits dans une des langues principales;
2. de *brèves communications;*
3. informera ses lecteurs des événements marquants de la vie scientifique, donnera des *comptes rendus concernant les récentes publications, les congrès et les assemblées.*

Die Experientia stellt sich die Aufgabe:

1. durch *zusammenfassende Originalartikel* in einer der wissenschaftlichen Hauptsprachen von Autoren aus verschiedenen Ländern über Forschungsergebnisse berichten zu lassen, die im Vordergrund des Interesses stehen;
2. kurze *Mitteilungen* aufzunehmen;
3. durch Besprechung neuerschienener *Bücher*, durch Referate über *Kongresse* und *Versammlungen* sowie durch andere Mitteilungen über die bedeutendsten Ereignisse des naturwissenschaftlichen Lebens zu informieren.

Experientia si propone di pubblicare:

1. *articoli originali riassuntivi*, in una delle principali lingue usate dalla scienza, ad opera di autori di diversi paesi, su risultati scientifici di grande interesse;
2. *brevi comunicazioni;*
3. *recensioni di nuovi libri, relazioni di congressi e riunioni*, come pure altre comunicazioni su importanti avvenimenti nel campo delle scienze naturali.

The aim of Experientia is:

1. to publish comprehensive articles embodying the results of recent scientific research. These will be written in one of the principal scientific languages and contributed by authors in various countries;
2. to publish *brief reports;*
3. to give information about the most important events in natural science by means of *reviews of the latest books, reports on congresses and meetings*, as well as through other communications.

**EXPER.**


L'Experientia paraît le 15 de chaque mois. Vente et abonnement dans toutes les librairies suisses et étrangères, ou directement chez l'éditeur. Abonnement pour un an Fr. 32.– pour la Suisse; pour l'étranger fr. 38.–. Ces prix s'entendent en francs suisses.

Adresser toute correspondance touchant la rédaction de l'Experientia exclusivement à l'éditeur soussigné.

Dernier délai d'admission pour les manuscrits: 35 jours avant la parution, c'est-à-dire le 10 du mois pour le numéro du mois suivant.

Les auteurs recevront gratuitement, s'ils le désirent, 50 tirés à part de format 14,5 sur 21 cm, sans couverture. Pour le prix d'un nombre plus grand et pour la couverture s'adresser à l'éditeur. Les tirages à part doivent être commandés *avant* l'impression du périodique.

Prix pour les annonces: $^1/_1$ page fr. 220.–, ½ page fr. 132.–, ¼ page fr. 77.–. Placements spéciaux: prix sur demande. Demandes à l'éditeur.

L'Experientia est imprimée en Suisse.

*Editions Birkhæuser S.A., Bâle 10 (Suisse), Elisabethenstrasse 15*
*Tél. 249800; adresse télégraphique: Edita Bâle*

Die Experientia erscheint am 15. jedes Monats und kann im In- und Auslande durch jede Buchhandlung oder direkt vom Verlag bezogen werden. In Algerien, Belgien, Dänemark, Deutschland, Finnland, Frankreich, Italien, Luxemburg, Marokko, Norwegen, Österreich, Portugal, Schweden nehmen auch die Postämter Bestellungen entgegen. Der Abonnementspreis beträgt in der Schweiz Fr. 32.–, im Ausland sFr. 38.–.

Alle Zuschriften an die Redaktion sind ausschliesslich an den Verlag zu richten. Redaktionsschluss 35 Tage vor Erscheinungstermin, das heisst am 10. des Monats für den folgenden Monat.

Die Autoren erhalten auf Wunsch 50 Gratisseparata im Format 14,5 ×21 cm, ohne Umschlag. Die Kosten für weitere Separata und für Umschläge sind beim Verlag zu erfragen. Separata sind *vor* dem Druck der Zeitschrift zu bestellen.

Insertionspreise: $^1/_1$ Seite Fr. 220.–, ½ Seite Fr. 132.–, ¼ Seite Fr. 77.–; für Vorzugsseiten besondere Vereinbarung. Inseratenannahme durch den Verlag.

Die Experientia wird in der Schweiz gedruckt.

*Verlag Birkhäuser AG., Basel 10 (Schweiz), Elisabethenstrasse 15*
*Tel. 249800; Telegrammadresse: Edita Basel*

Experientia esce al 15 di ogni mese e può esser richiesta ad ogni libreria svizzera o estera, o anche direttamente alla casa editrice. L'abbonamento annuo è di fr. 32.– per la Svizzera; all'estero fr. 38.–. I prezzi vanno intesi valuta svizzera.

Tutti gli invii alla redazione di Experientia vanno indirizzati esclusivamente alla sottoindicata casa editrice.

La redazione di ogni fascicolo si chiude 35 giorni prima del termine di pubblicazione, cioè al 10 del mese, per il mese seguente.

Gli autori ricevono, su desiderio, 50 estratti del formato 14,5 ×21 cm, senza copertina. Il prezzo degli estratti in più e della copertina viene indicato, su richiesta, dalla casa editrice. Gli estratti vanno ordinati *prima* della stampa della Rivista.

Prezzi per annunci: $^1/_1$ pag. fr. 220.–, ½ pag. fr. 132.–, ¼ pag. fr. 77.–; per pagine speciali, accordi da stabilire. Gli annunci sono da inviare alla casa editrice.

Experientia si stampa in Isvizzera.

*Casa editrice Birkhæuser S.A., Basilea 10 (Svizzera), Elisabethenstr. 15*
*Tel. 249800; Indirizzo telegrammi: Edita Basilea*

Experientia is published on the 15th of every month and can be obtained in any country through the booksellers or from the publishers. The annual subscription price by inland mail is fr. 32.–; other countries fr. 38.–. Prices in Swiss currency. All communications to the editors should be addressed to the publishers. All manuscripts for publication in a given number must be in the hands of the publishers on the 10th of the preceding month.

The authors receive, on request, 50 reprints 14,5 ×21 cm without cover free of charge. For the prices of additional reprints and covers, inquiries should be addressed to the publishers. Reprints must be ordered *before* the number is printed.

Prices for advertising: $^1/_1$ page fr. 220.–, ½ page fr. 132.–, ¼ page fr. 77.–. Advertisements should be sent to the publishers.

*Published by Birkhäuser Ltd., Basle 10 (Switzerland), Elisabethenstr. 15*
*Phone 249800; Telegrams: Edita Basle*

Printed in Switzerland / E. Birkhäuser & Cie., Basel



*Verzeichnis der Inserenten – Liste des annonceurs – List of Advertisers – Experientia X/1*

Academic Press Inc., New York
Ciba AG., Basel
Ganz & Co., Zürich
Long Island Ass., New York
Lonza AG., Basel
Sandoz AG., Basel
Steiner & Co., Basel
Trüb, Täuber & Co. AG., Zürich
Verlag Chemie GmbH., Weinheim

64-06678


# LONZA

ELEKTRIZITÄTSWERKE
UND
CHEMISCHE FABRIKEN
AKTIENGESELLSCHAFT
BASEL

**Calcium-Carbid** für Beleuchtungs-, Heiz- und Schweisszwecke

**Metallegierungen:** Ferrosilicium, Reinsilicium, Silico-Aluminium, Silico-Aluminium-Mangan — Graphit

**Künstliche Schleifmittel:** Siliciumcarbid, Lonsicar (Siliciumcarbid) für Hartbeton

**Stickstoff-Dünger:** Kalksalpeter, Ammonsalpeter, Ammonsulfat, Kalkstickstoff

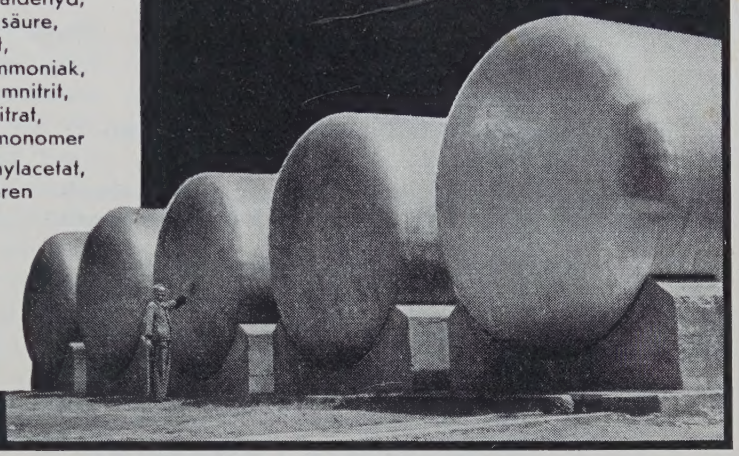
**Komplex-Dünger:** Nitrophosphat, Nitrophosphatkali, Volldünger, Composto Lonza

**Chemische Produkte:** Formaldehyd, Acetaldehyd, Crotonaldehyd, Paraldehyd, Essigsäure, Essigsäureanhydrid, Natriumacetat, Dicyandiamid, Guanidinnitrat, Ammoniak, Salpetersäure, Nitriersäure, Natriumnitrit, Natriumnitrat, Harnstoff, Ammonnitrat, Nitrobenzol, Anilinöl, Vinylacetat monomer

**Organische Lösungsmittel:** Aceton, Aethylacetat, Methyl- und Butylalkohol und deren Acetate, Speziallösungsmittel

**Cellulose-Acetat**

**Vinylharze:** Polyvinylchlorid, Polyvinylacetat, Polyvinylalkohol, Mischpolymerisate



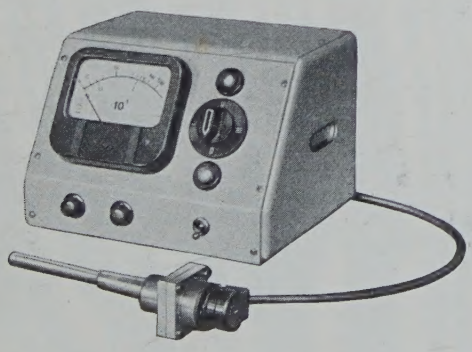


**Gerätebau-Anstalt** GAB **Balzers (FL.)**

## Vakuum-Messgeräte

kontinuierlich anzeigend zwischen

$10^{-5}$ bis 1 mm Hg



Vollkommen unempfindlich gegen Lufteinbrüche in eingeschaltetem Zustand. Einstellzeit max. 2 Sek. Eingebauter Umschalter für Anschluss von 4 Messzellen, Gerät auch als „Leak-detector" verwendbar.

**Verkaufsorganisation**

**Trüb, Täuber & Co. AG. Zürich**
Fabrik elektr. Meßinstrumente und wissenschaftl. Apparate



# VIRUSES

Cold Spring Harbor Symposia on Quantitative Biology
**Volume XVIII (1953) xvi + 302 quarto pag. with numerous figures**

Authoritative reviews of research dealing with quantitative problems, presented in 42 papers and edited discussions. Problems considered are: states of viruses and transition from one state to another; genetics; structure; biochemical studies; host-controlled variations; and tissue cultures.

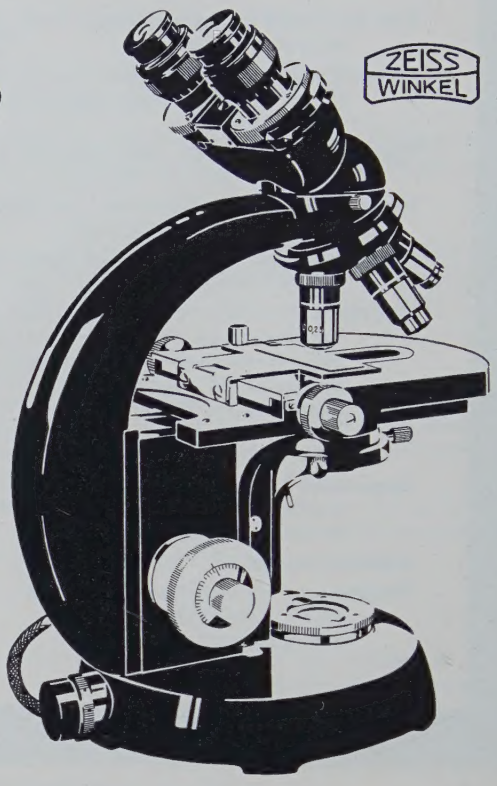
Previous volumes still available: IX (1941) Genes and Chromosomes; XI (1946) Heredity and Variation in Microorganisms; XII (1947) Nucleic Acids and Nucleoproteins; XIII (1948) Biological Applications of Tracer Elements; XIV (1949) Amino Acids and Proteins; XV (1950) Origin and Evolution of Man; XVI (1951) Genes and Mutations; XVII (1952) The Neuron.

Prices: Volumes IX–XV, $7; XVI, $9; XVII and XVIII, $8. Volumes IX, XV, XVI and XVIII: any two, $14; any three, $20; all four, $26. Postage extra: domestic, 25 cents; foreign, 50 cents per volume.

# NEUES **ZEISS**-WINKEL STANDARD-MIKROSKOP GF 625

**Das Ergebnis einer hundertjährigen Tradition !**

- **Lichtstarke Einbaubeleuchtung (Köhlersches Prinzip) für subjektive Beobachtung und Mikrophotographie**
- **Tiefliegende, koaxial gelagerte Triebknöpfe für Grob- und Feineinstellung**
- **Grosser Kreuztisch mit koaxial gelagerten, links- und rechtsseitig angebrachten Triebknöpfen**
- **Vollkommener Präparatschutz durch gefederte Fassung der Objektive**
- **Vergrösserungswechsler für die Okulare (ohne Okularwechsel)**
- **Schlittenrevolver für fünf Objektive**
- **Vereinigter Hellfeld-Phasenkontrast-Dunkelfeld-Kondensor**
- **Neue Achromate und Neofluare mit dezimalgeometrischer Abstufung der Vergrösserungen**




**Auskunft und Vorführung durch die Generalvertretung für die Schweiz:**

**GANZ & Co**
BAHNHOFSTR. 40
TEL.(051) 23 97 73
*Zürich*

EXPERIENTIA

Vol. X - Fasc. 1 Pag. 1–40 15. I. 1954

# Some Stereochemical Aspects of Polyenes

By L. ZECHMEISTER, Pasadena, Calif.[1]

*Introduction*

One hundred and thirty-five years ago a Marseille pharmacist, named POUTET[2], conducted a remarkable experiment. He shook olive oil with a reagent, obtained by dissolving mercury in excess nitric acid, and observed a few hours later that the oil had solidified. Some other vegetable oils remained liquid upon a similar treatment. POUTET thus found a suitable method for the detection of falsifications in commercial olive oil. Of course, he could not have been aware of the fact that he had performed a pioneer experiment in stereochemistry, half a century before VAN T'HOFF and LEBEL disclosed their fundamental ideas.

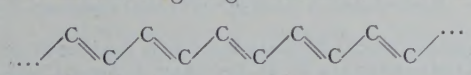
Since olive oil is rich in glycerides including unsaturated fatty acids and since the molecules of most such acids contain at least one double bond in *cis* configuration, POUTET's experiment must be rated as a *cis* → *trans* rearrangement, termed "elaidinization" in modern fat chemistry.

*cis*-Configuration. *trans*-Configuration[3].

As is well known, VAN T'HOFF's concept of *cis-trans* isomerism was too revolutionary to be accepted without contradiction. Even after a lapse of ten years ANSCHÜTZ[4], a leading organic chemist, rejected the spatial character of the maleic acid–fumaric acid relationship; he assumed that such a "slight" variation could not possibly explain the very different behavior of the two acids.

A similar scepticism was voiced not such a long time ago with reference to the existence of stereoisomeric forms of polyenes.

Although the term "poly-ene" would *a priori* include any compound whose molecules contain more than one double bond in an open chain, the present use of this term is rather generally restricted to "conjugated" systems of alternating single and double bonds:



Whereas many properties of compounds of another type, containing "isolated" double bonds in an open chain, can be understood as the sum of the contributions of the individual double bonds, in contrast, essentially new features appear when the system becomes conjugated. Some authors have suggested[1] that the electrons are "smeared" along such systems, depriving each C=C group of most of its double bond character. This would exclude the existence of *cis* forms. Although this assumption seemed to be confirmed by the almost exclusive occurrence of the all-*trans* configuration both in natural and synthetic polyenes, it was pointed out by KUHN[2] that in the case of the Orleans pigment, bixin, Nature herself has provided us with an example of a *cis* polyene, the character of which has been clarified by KARRER[3].

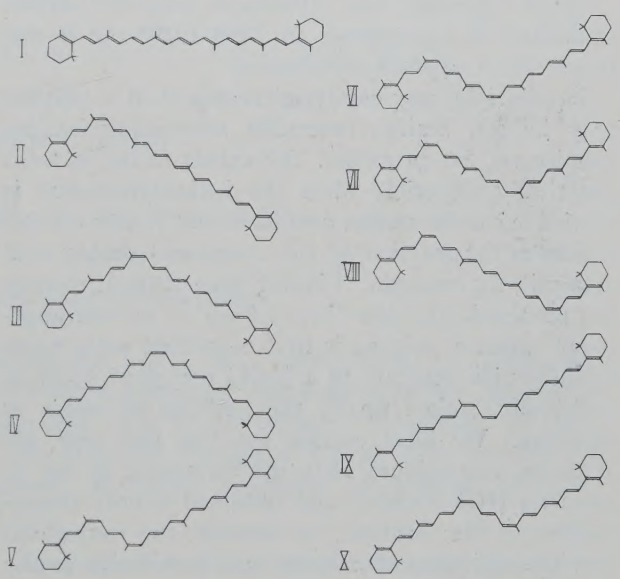


Fig. 1.–A few carbon skeleton models of stereoisomeric β-carotenes: I, all-*trans*; II–IV, mono*cis*; and V–X, di*cis* forms.

Today we know that polyenes are generally subject to *cis-trans* isomerization; and a few carbon skeleton models of stereoisomeric β-carotenes (Fig. 1) will suffice to illustrate the spatial versatility of such molecules.

[1] California Institute of Technology, Pasadena.

[2] J. J. E. POUTET, Ann. chim. phys. [2] *12*, 58 (1819).

[3] The prefixes, *cis* and *trans*, were introduced by A. BAEYER, Liebigs Ann. Chem. *245*, 103 (1888).

[4] R. ANSCHÜTZ, Liebigs Ann. Chem. *239*, 161 (1887).

[1] G. WITTIG and W. WIEMER, Liebigs Ann. Chem. *483*, 144 (1930).

[2] R. KUHN, in Freudenbergs Stereochemie (Fr. Deuticke, Leipzig-Wien, 1933), p. 913.

[3] P. KARRER, A. HELFENSTEIN, R. WIDMER, and TH. B. VAN ITALLIE, Helv. chim. Acta *12*, 741 (1929).

### *Methods of cis-trans Isomerization*

The experimental procedures leading from the ordinary or all-*trans* form of a polyene to its *cis* isomers are in principle identical with those having effect on mono- or di-unsaturated compounds. However, instead of a simple product, a complicated mixture of stereoisomers is obtained in which unchanged all-*trans* molecules may or may not predominate. The most frequently applied thermic and photochemical treatments are: keeping a solution at room temperature ("spontaneous stereoisomerization"), refluxing solutions, melting crystals, illumination of solutions with light of suitable wave length or exposure to sunshine. Among the stereo-catalytic methods available at room temperature the very effective treatment with iodine should be emphasized.

It was observed by ANSCHÜTZ[1] in 1878 that the silver salts of both maleic and fumaric acids when reacted with ethyl iodide yielded the same ester, viz. ethyl fumarate. Subsequently, ANSCHÜTZ found that a slight iodine content of the ethyl iodide used in his first experiments had been responsible for this surprising identity, in the sense that the iodine had catalytically converted the maleic into fumaric acid. Silver maleate, upon interaction with iodine-free ethyl iodide, gave exclusively the maleic ester.

Iodine catalysis was introduced into the stereochemistry of $C_{40}$-polyenes in 1938–1939[2] and it was found that it requires illumination.

In principle, any catalytic treatment of a polyene may induce, besides reversible stereoisomerization, also irreversible processes. The extent of the latter is practically negligible when the iodine treatment is carried out under proper conditions but it may involve a substantial fraction of the compound during acid catalysis, for example. It is still more difficult to steer the process in the direction of *trans* → *cis* rearrangements when a polyene is first converted with boron trihalogenide etherate to a (dark) complex[3] which is eventually broken up by the addition of water or methanol. We have carried out this last step, for example, one minute after the formation of the β-carotene (III) complex and obtained mainly stereoisomers of the carotene; in contrast, the conjugated system underwent shortening and destruction in the course of a longer standing of the complex[4]. Dehydro-β-carotene (IV) is so sensitive that even a one-minute standing of its $BF_3$-complex results in the formation of conversion products while no stereoisomers appear[5].

Theoretically, it is to be expected that any stereoisomerization would result in the formation of a (quasi-)equilibrium mixture containing all possible "members of the stereoisomeric set". Experiment has shown, however, that in most instances only a few, viz. 3 to 10, molecular forms are preferred and that on the usual laboratory scale most of the possible stereoisomers escape detection.

A successful study of *cis-trans* isomeric polyene mixtures requires thus the solution of the following two problems: (1) the stereoisomeric mixtures must be resolved, and (2) each pure, individual compound must be proven to represent a stereoisomer of the starting material.

Since polyenes are not volatile, the only means for their effective separation is chromatographic analysis. It has been found in this class of compounds that the adsorption affinities are as highly dependent on the spatial configuration as on the chemical structure. Hence, differences in the molecular shape do permit clear separations on the chromatographic column. In order to prove that a zone truly contains a stereoisomer of a given polyene and nothing else, it is eluted, submitted to iodine catalysis and the solution chromatographed again. The all-*trans* zone thus obtained is then mixed with an authentic sample of the same substance and developed on a column ("mixed chromatogram test"). Non-separation indicates identity which should be confirmed by a comparison of the spectra.

Using the methods just outlined, a number of *cis* carotenoids have been prepared, several of them in the crystalline state, and their physical, chemical and biological characteristics (if any) have been determined. Although this development encountered considerable scepticism at first[1], most of our methods and results now appear to have been generally accepted.

### *Types of Polyenes Investigated*

Although a variety of polyene systems still remains to be explored by the stereochemist, marked progress has been made in the following four classes of compounds[2]:

(a) *Non-isoprenic conjugated systems with aromatic end groups:* α, ω-Diphenylpolyenes[3]:

$$C_6H_5 \cdot (CH = CH)_n \cdot C_6H_5$$

($n = 1$: stilbene; $n = 2$: diphenylbutadiene; $n = 3$: diphenylhexatriene; and $n = 4$: diphenyloctatetraene).

[4] R. ANSCHÜTZ, Ber. dtsch. chem. Ges. *11*, 1644 (1889); *12*, 2282 (1879).

[2] L. ZECHMEISTER and P. TUZSON, Nature *141*, 249 (1938); Biochem. J. *32*, 1305 (1938); Ber. dtsch. chem. Ges. *72*, 1340 (1939).

[3] G. N. LEWIS and G. T. SEABORG, J. Amer. Chem. Soc. *61*, 1886 (1939). – H. H. STRAIN, J. Amer. Chem. Soc. *63*, 3448 (1941).

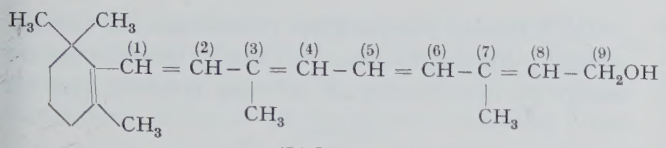
[4] L. WALLCAVE, J. LEEMANN, and L. ZECHMEISTER, Proc. Nat. Acad. Sci. (U.S.A.), *39*, 604 (1953).

[5] L. WALLCAVE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 4495 (1953).

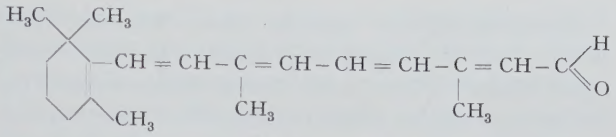
[1] Cf. P. KARRER and J. RUTSCHMANN, Helv. chim. Acta *27*, 1684 (1944).

[2] General survey: L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

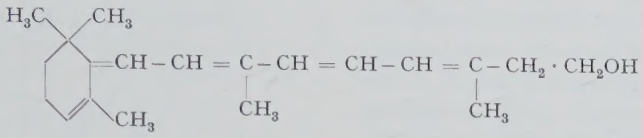
[3] L. ZECHMEISTER and W. H. MCNEELY, J. Amer. Chem. Soc. *64*, 1919 (1942). – L. ZECHMEISTER and A. L. LEROSEN, J. Amer. Chem. Soc. *64*, 2755 (1952). – A. SANDOVAL and L. ZECHMEISTER, J. Amer. Chem. Soc. *69*, 553 (1947). – J. H. PINCKARD, B. WILLE, and L. ZECHMEISTER, J. Amer. Chem. Soc. *70*, 1938 (1948). – Unpublished studies, in collaboration with K. LUNDE and with J. H. PINCKARD.



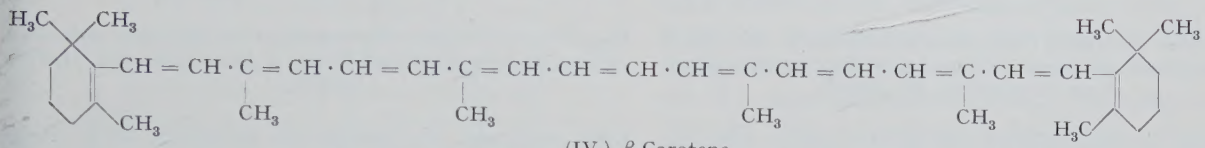
(I.) Vitamin A



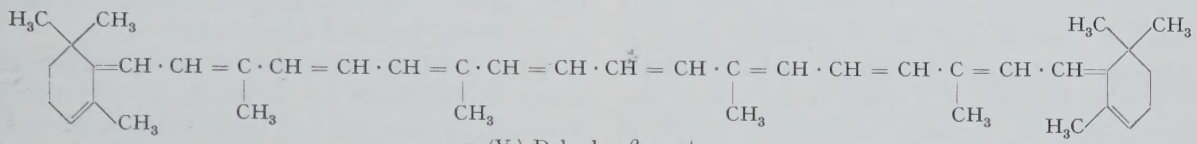
(II.) Retinene



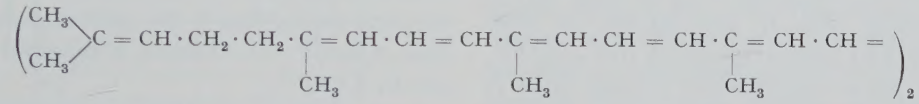
(III.) Retrovitamin A



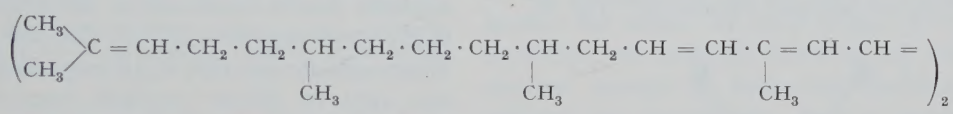
(IV.) β-Carotene



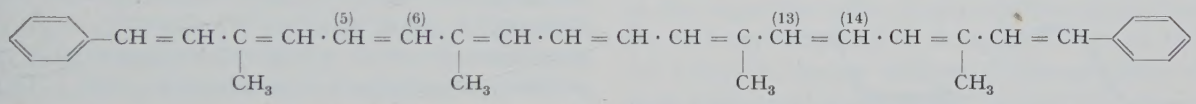
(V.) Dehydro-β-carotene



(VI.) Lycopene



(VII.) Phytofluene (probable formula)



(VIII). Diphenyl-3, 7, 12, 16-tetramethyl-octadecanonaene

Symmetrical azines[1]:

$$C_6H_5 \cdot (CH=CH)_n \cdot CH=N-N=CH \cdot (CH=CH)_n \cdot C_6H_5$$

($n = 1$: cinnamalazine; and $n = 2$: phenylpentadienalazine).

(b) *Non-isoprenic conjugated systems with heterocyclic end groups:* a representative of the cyanine dyes[2].

(c) *Isoprenic conjugated systems with aliphatic or hydroaromatic end groups:* $C_{20}$–$C_{25}$-Polyenes: vitamin A and related compounds[3], retinene[4], retro-vitamin A[5] (Formulas I–III), etc.; methylbixin[1]. $C_{40}$-Polyenes: carotenoid pigments[2] and some colorless, fluorescent polyenes such as phytofluene[3] (Formulas IV–VII).

(d) *Isoprenic conjugated systems with aromatic end groups:* KARRER's synthetic compounds[4] (Formula VIII).

*Steric Hindrance and Number of cis-trans Isomers*

The formulas (I–VIII) represent all-*trans* compounds. Provided that each of their *trans* double bonds is able to assume *cis* configuration, numerous steric forms can be expected, for example, 16 for vitamin A, 272 for β-carotene, and 1056 for lycopene.

[1] J. DALE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 2379 (1953).

[2] L. ZECHMEISTER and J. H. PINCKARD, Exper. *9*, 16 (1953).

[3] CH. D. ROBESON and J. G. BAXTER, J. Amer. Chem. Soc. *69*, 136 (1947). – J. D. CAWLEY, CH. D. ROBESON, L. WEISLER, E. M. SCHANTZ, N. D. EMBREE, and J. G. BAXTER, Science *107*, 346 (1948). – W. GRAHAM, D. A. VAN DORP, and J. F. ARENS, Rec. Trav. chim. Pays-Bas *68*, 609 (1949). – W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295, 3807 (1952); *75*, 1050 (1953).

[4] R. HUBBARD, R. I. GREGERMAN, and G. WALD, J. gen. Physiol. *36*, 415 (1953).

[5] W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295 (1952).

[1] L. ZECHMEISTER and R. B. ESCUE, J. Amer. Chem. Soc. *66*, 322 (1944).

[2] General survey: L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

[3] F. J. PETRACEK and L. ZECHMEISTER, J. Amer. Chem. Soc. *74*, 184 (1952).

[4] C. H. EUGSTER, C. F. GARBERS, and P. KARRER, Helv. chim. Acta *35*, 1179 (1952). – C. F. GARBERS, C. H. EUGSTER, and P. KARRER, Helv. chim. Acta *36*, 562 (1953).

Three-dimensional models show, however, that *trans* → *cis* rearrangements cannot take place with equal facility around each double bond, considering the presence of some geometrical hurdles. Three types of such "steric hindrance" have been under discussion so far.

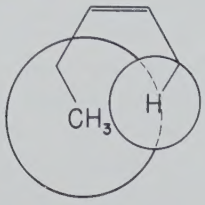



Fig. 2.–Steric hindrance: Overlapping of a hydrogen atom and a methyl group in *cis* isoprenic polyenes. [From: Fortschr. Chem. organ. Naturstoffe *3*, 203 (1939).]

Firstly, it was pointed out by PAULING[1] with reference to isoprenic polyenes that when a carbon atom adjacent to a double bond carries a methyl group, a considerable overlapping of this group and a hydrogen atom is to be expected as a result of *trans* → *cis* isomerization (Fig. 2). Secondly, according to LEWIS *et al.*[2] two H-atoms attached to the two rings in *cis*-stilbene will overlap (Fig. 3); and thirdly, we have shown for terminal-*cis* diphenylpolyenes that a hydrogen atom of the ring, in opposition to the side chain, is spatially hindered by the latter; thus an approximately planar configuration becomes impossible[3] (Fig. 4).

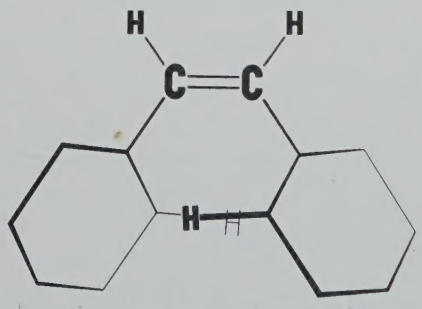



Fig. 3.–Steric hindrance: Overlapping of two ring-hydrogen atoms in *cis*-stilbene. [From: J. Amer. Chem. Soc. *62*, 2973 (1940).]

During the last few years several authors have discussed the existence or non-existence of sterically hindered *cis* polyenes. Recently, several experimental studies have shown that none of the three hindrance types mentioned constitutes an absolute block. Certain hindered *cis* forms do exist and they are not necessarily thermolabile in spite of their relatively high energy content. It should be noted, however, that "hindered" configurations rarely appear upon iodine catalysis; on the contrary, they seem to be very iodine sensitive and disappear from the solution when catalyzed.

[1] L. PAULING, Fortschr. Chem. organ. Naturstoffe *3*, 203 (1939).
[2] G. N. LEWIS, T. T. MAGEL, and D. LIPKIN, J. Amer. Chem. Soc. *62*, 2973 (1940).
[3] L. ZECHMEISTER and A. L. LEROSEN, J. Amer. Chem. Soc. *64*, 2755 (1942).

The following two practical routes have led to some sterically hindered forms: (1) direct introduction of energy by illuminating an all-*trans* polyene; and (2)

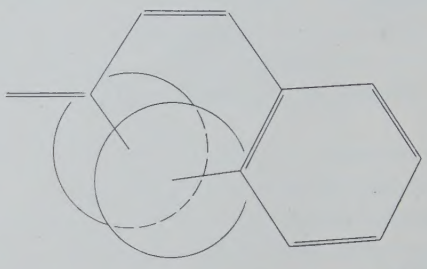


Fig. 4.–Steric hindrance: Overlapping of two hydrogen atoms in a terminal *cis*-diphenylpolyene. [From: J. Amer. Chem. Soc. *64*, 2755 (1942).]

total synthesis *via* the partial reduction of a triple bond (or of a cumulene structure) located at the site of the desired hindered double bond.

In the non-hindered system of maleic-fumaric acids it was demonstrated by WARBURG in 1919 that the *trans* acid is converted into the *cis* form by ultraviolet illumination. An early example in the field of hindered ethylene derivatives is the photochemical conversion, *trans*-stilbene → *cis*-stilbene, as reported by STOERMER[1] and by LEWIS[2].

In the stilbene and diphenylbutadiene molecules all aliphatic double bonds are of "hindered" nature, and in diphenylhexatriene all but that one in the center. When a dilute solution of all-*trans*-diphenylbutadiene was kept in diffuse daylight, rearrangement to a hindered *cis* form took place; however, the all-*trans* configuration was recovered almost quantitatively upon iodine catalysis (Fig. 5)[3]. Recently, a *cis* cinnamalazine for which a hindered configuration is very probable was obtained, in collaboration with J. DALE, by prolonged exposure of *trans* solutions to intense sunshine or by melting crystals[4]. The *cis*-diphenylbutadienes had been prepared by the partial reduction of the corresponding acetylene derivatives half a century ago (STRAUS)[5]. In our laboratory J. H. PINCKARD and K. LUNDE have obtained some hindered *cis* forms of diphenyl-octatetraene and -hexatriene by direct isomerization of the respective all-*trans* compounds (unpublished).

In the field of isoprenic polyene systems the first case of a hindered *cis* isomer was reported by OROSHNIK[6]. In the class of carotenoid pigments the well-

[1] R. STOERMER, Ber. dtsch. chem. Ges. *42*, 4865 (1909). – R. STOERMER and L. PRIGGE, Liebigs Ann. Chem. *409*, 20 (1915).
[2] G. N. LEWIS, T. T. MAGEL, and D. LIPKIN, J. Amer. Chem. Soc. *62*, 2973 (1940). – G. N. LEWIS and J. BIGELEISEN, J. Amer. Chem. Soc. *65*, 2102, 2107 (1943).
[3] A. SANDOVAL and L. ZECHMEISTER, J. Amer. Chem. Soc. *69*, 553 (1947). – J. H. PINCKARD, B. WILLE, and L. ZECHMEISTER, J. Amer. Chem. Soc. *70*, 1938 (1948).
[4] J. DALE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 2379 (1953).
[5] F. STRAUS, Liebigs Ann. Chem. *342*, 190 (1905).
[6] W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295, 3807 (1952); *75*, 1050 (1953).

known total-synthetic work[1] carried out by the schools of KARRER in Zürich and INHOFFEN in Braunschweig made it possible to prepare, *via* acetylene derivatives, all types of *cis* isomers, including some

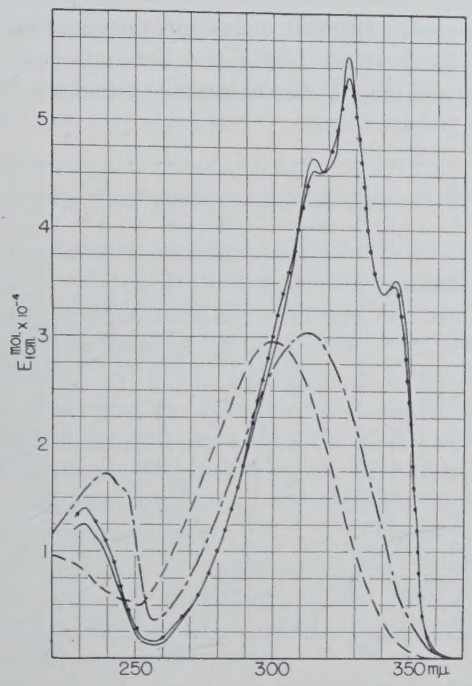



Fig. 5.–Molecular extinction curves of stereoisomeric diphenylbutadienes, in hexane: ———, *trans-trans*; – – –, *cis-trans*; and – – – – –, *cis-cis*; –·–·–·–, after iodine catalysis of any of the three stereoisomers. [From: J. Amer. Chem. Soc. *70*, 1938 (1948).]

hindered forms, with determined absolute configurations. The importance of similar synthetic compounds, especially of KARRER's hindered isoprenic polyenes containing aromatic end groups, in the evaluation of some spectral phenomena will be indicated below[2].

### *Steric Configuration and the Main Spectral Band*

While chromatography constitutes a unique tool for the separation and isolation of *cis-trans*-isomeric polyenes, spectroscopy is the choice method of rapid characterization of a pure, individual spatial form. Although the exact relationship between the molecular shape and spectrum is not yet fully understood, some interesting correlations have been secured.

The extinction curve of a polyene contains, first of all, a main band, located within or near the visible region and showing more or less fine structure. According to classical electromagnetic theory it represents the vibration of mobile "unsaturation" electrons along the whole length of the conjugated system. Resonance stabilization at the single bonds keeps an all-*trans* chromophore approximately coplanar[1]. Within a given stereoisomeric set the all-*trans* compound occupies a privileged position, in the sense that its main spectral band shows a longer wave length maximum and a higher extinction value than the band of any *cis* isomer. Thus, the straight molecular form of an all-*trans* pigment brings about the strongest possible color within the set. Since the all-*trans* configuration is by far preponderant in the vegetable kingdom, Nature when producing color makes the best possible use of a given conjugated system. The older concept that the color of a polyene is determined only by the length of the chromophore had to be abandoned, since it did not envisage the profound influence of the steric configuration on the spectrum.

Let us now imagine the following experiment: Starting from an all-*trans* polyene we perform a stepwise *trans* → *cis* rearrangement, proceeding from one double bond to the next. We assume that each such step will cause some loss of fine structure in the main band and a certain displacement of the main maximum towards shorter wave lengths. No theory is available at the present time on the basis of which one could predict the extent of these shifts. An experimental approach was possible, however, by taking the extinction curves of numerous chromatographically purified *cis* carotenoids. It was observed that in many instances the location on the wave length scale of the respective main maxima differed from that of the all-*trans* compound by 5 mμ or 10 mμ or 15 mμ (± 1 mμ; in hexane solution). Although these data were interpreted by assuming, respectively, mono*cis*, di*cis*, and tri*cis* configurations, it was stressed nine years ago that "nothing definite is known about the additivity of spectral differences and their dependence on the relative position of the double bond concerned"[2]. Hence, considerable aberrations from the observed "regularity" had to be expected in special instances.

Since the quoted statement was made, some pertinent new spectral data have appeared in the literature referring to such *cis* compounds whose configurations had been determined by total synthesis. INHOFFEN and his colleagues[3] reported that their central mono-*cis*-β-carotene (carbon skeleton: Fig. 1; there Model IV) differed from all-*trans*-β-carotene by 3 mμ only in the location of the main maximum. This behavior might be explained by the unique structural position

[1] P. KARRER and C. H. EUGSTER, C. r. Acad. Sci. *250*, 1920 (1950), and numerous papers in the Helv. chim. Acta. – H. H. INHOFFEN, F. BOHLMANN, K. BARTRAM, and H. POMMER, Chem. Z. *74*, 285 (1950), and numerous papers in Liebigs Ann. Chem. Cf. also N. A. MILAS, P. DAVIS, I. BELIČ, and D. A. FLEŠ, J. Amer. Chem. Soc. *72*, 4844 (1950).

[2] *Addendum:* C. F. GARBERS and P. KARRER, Helv. chim. Acta *36*, 828 (1953), have synthesized, quite recently, several hindered *cis* lycopenes. These could not be obtained in crystalline form and have been characterized on the basis of spectral readings. Their spectra are very similar to some observed in the series of poly*cis*-lycopenes.

[1] L. PAULING, Fortschr. Chem. organ. Naturstoffe *3*, 203 (1939). – L. ZECHMEISTER, A. L. LEROSEN, W. A. SCHROEDER, A. POLGÁR, and L. PAULING, J. Amer. Chem. Soc. *65*, 1940 (1943).

[2] L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

[3] H. H. INHOFFEN, F. BOHLMANN, K. BARTRAM, G. RUMMERT, H. POMMER, F. WESTPHAL, and G. LINHOFF, Liebigs Ann. Chem. *570*, 54 (1950).

of the central double bond, the only one which is not connected with C-methyl groups.

On the other hand, it was observed by KARRER *et al.*[1] when studying the system represented in formula (VIII) that the rearrangement, all-*trans* → 5,6-(hindered) mono*cis*, caused an extraordinarily large spectral shift. At the same time the curve flattened out markedly, although the fine structure did not disappear. However, the presence of two hindered *cis* double bonds (5,6- and 13,14-) did practically demolish the fine structure.

In the much shorter diphenylbutadiene system[2] such total loss of fine structure appeared upon a single rearrangement from all-*trans* to hindered *cis* (Fig. 4).

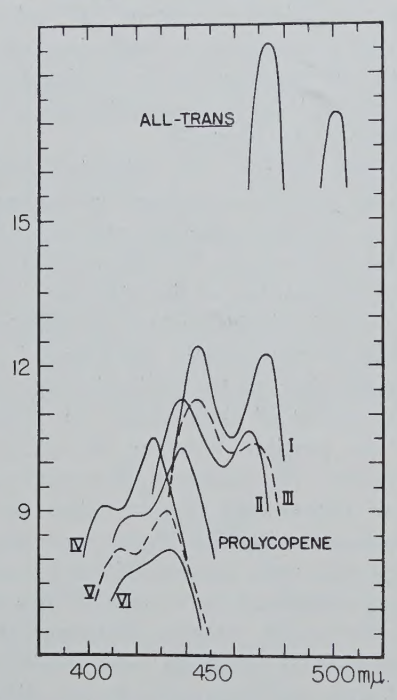



Fig. 6.–Molecular extinction curves, in hexane, of all-*trans*-lycopene and seven poly*cis* lycopenes at their main maxima. [From: J. Amer. Chem. Soc. *69*, 1930 (1947).]

These relationships are also illustrated by some observations made on naturally occurring *cis* carotenoids[3]. Evidently, under the influence of special genetic factors[4], some plant varieties deviate from the "all-*trans* rule" and steer the pigment biosynthesis towards *cis* configurations. Thus, we were able to isolate from the berries of *Pyracantha angustifolia* several "poly-*cis*" lycopenes in crystalline form[1]. When inspecting their spectral curves as given in Figure 6, one would not assume *a priori* that they all refer to the same chemical constitution. The sharp dependence of the extent of fine structure on the molecular form is remarkable indeed. The main band of central mono *cis*-lycopene (Fig. 7) lies between those of the all-*trans* and the poly*cis* forms.

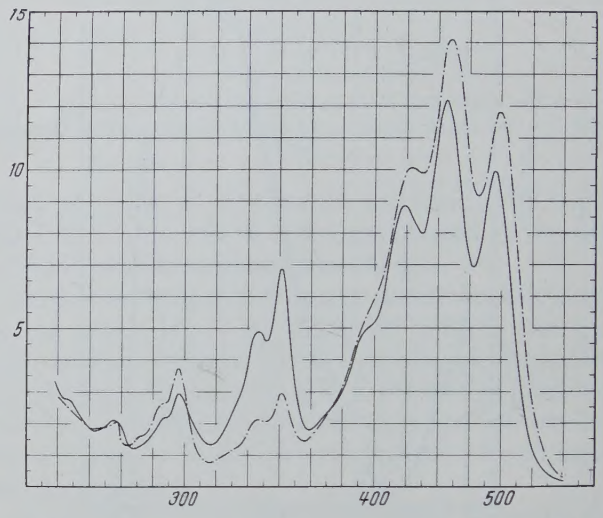



Fig. 7.–Molecular extinction curve, in hexane, of neolycopene A (very probably central mono*cis*-lycopene): ———, before iodine catalysis; and –·–·–, after iodine catalysis. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

The poly*cis* compound "prolycopene" when catalyzed with relatively large amounts of iodine (1% of the pigment) yielded the expected stereochemical equilibrium mixture almost instantaneously, in which all-*trans*-lycopene (V) was preponderant (Fig. 8). However,

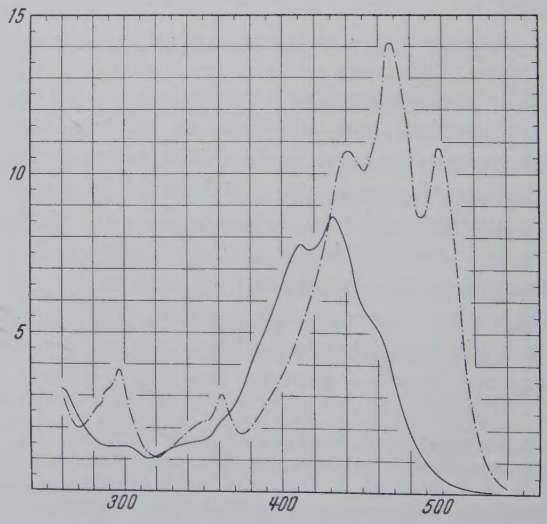



Fig. 8.–Molecular extinction curve, in hexane, of a poly*cis* lycopene: ———, before iodine catalysis; and ·–·–·–, after iodine catalysis. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

[1] C. H. EUGSTER, C. F. GARBERS, and P. KARRER, Helv. chim. Acta *35*, 1179 (1952). – C. F. GARBERS, C. H. EUGSTER, and P. KARRER, Helv. chim. Acta *35*, 1850 (1952); *36*, 562 (1953).

[2] A. SANDOVAL and L. ZECHMEISTER, J. Amer. Chem. Soc. *69*, 553 (1947). – J. H. PINCKARD, B. WILLE, and L. ZECHMEISTER, J. Amer. Chem. Soc. *70*, 1938 (1948).

[3] A. L. LEROSEN and L. ZECHMEISTER, J. Amer. Chem. Soc. *64*, 1075 (1942). – L. ZECHMEISTER and W. A. SCHROEDER, J. Amer. Chem. Soc. *64*, 1173 (1942).

[4] L. ZECHMEISTER and F. W. WENT, Nature (London) *162*, 847 (1948). – J. W. PORTER and F. P. ZSCHEILE, Arch. Biochem. *10*, 537, 547 (1946). – J. W. PORTER and R. E. LINCOLN, Arch. Biochem. *27*, 390 (1950). – H. H. TROMBLY and J. W. PORTER, Arch. Biochem. Biophys. *43*, 443 (1953).

[1] L. ZECHMEISTER and J. H. PINCKARD, J. Amer. Chem. Soc. *69*, 1930 (1947).

when the quantity of the catalyst was decreased 1000 times, for example, the process was slowed down and a chromatogram, taken after a few minutes' iodine treatment, contained a dozen stereoisomers whose spectral maxima were located between those of the catalyzed poly*cis* compound and the all-*trans* form. Two of the products, as judged on the basis of their spectra, seemed to contain even more *cis* double bonds than the starting material.

### *Steric Configuration and cis-Peak Effect*

It was observed ten years ago that when an ordinary (all-*trans*) carotenoid undergoes stereoisomerization, involving as described the decrease and shift of its main spectral band, at the same time a new maximum, the so-called "*cis*-peak" grows out in a shorter wave length region (Fig. 9)[1]. As a rule the *cis* forms of a given polyene having a *cis*-peak shows it at the same wave length; however, the height of the peak is subject to great variations and is a characteristic constant of each stereoisomeric form (Fig. 10). The distance as measured in m$\mu$ on the wave length scale between the *cis*-peak and the maximum of the main band is practically constant for a given conjugated system; it is shorter in shorter systems.

Fig. 9.–Molecular extinction curve of lycopene, in hexane: ———, all-*trans* form; – – – –, after refluxing for 45 min; and ·–·–·–, after iodine catalysis. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

According to PAULING *et al.*[2] the *cis*-peak arises from the oscillation of the electrons from the two ends of a conjugated system toward the center and from the center toward the two ends. The nature of this oscillation is such that it gives rise to no dipole moment and hence to no corresponding spectral band for the all-*trans* molecule. However, bent molecules do have a dipole moment that is perpendicular to the straight line between the ends of the conjugated system, instead of parallel to it. The central mono*cis* isomer would show the highest *cis*-peak of all. As a rough approximation the intensity of the *cis*-peak of a mono*cis* compound, for example, can be taken proportional to the square of the distance between the center of the conjugated system and the mid-point of the straight line between its two ends. Hence, spectral observations in the *cis*-peak region may well contribute to the steric diagnosis of a compound.

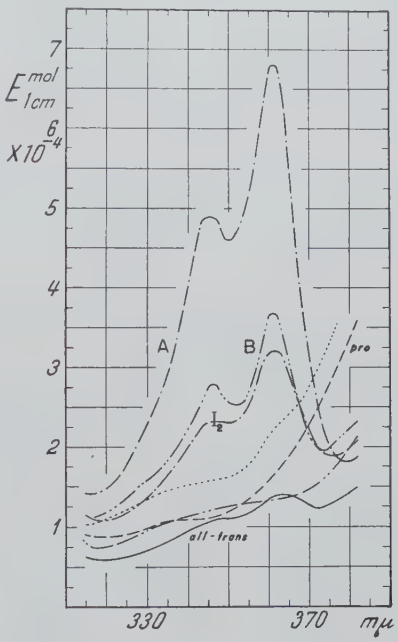



Fig. 10.–Molecular extinction curves in the *cis*-peak region, in hexane of several stereoisomeric lycopenes. ($I_2$ = quasi equilibrium mixture obtained upon iodine catalysis.) [From: J. Amer. Chem. Soc. *66*, 137 (1944).]

Since polyenes containing many *cis* double bonds have an overall straight molecular shape, comparable to that of the corresponding all-*trans* compound (Fig. 11), their spectral curves should not show any marked *cis*-peak. This postulate has been first verified by using naturally occurring "prolycopene" (Fig. 10). The influence of hindered *cis* configurations on the presence or absence of increased light absorption in the *cis*-peak region cannot yet be formulated in a general manner.

Recently, the *cis*-peak effect has also been observed when polyenes different from carotenoid pigments were used, such as phytofluene[1], diphenyloctatetraene[2]

[1] L. ZECHMEISTER and A. POLGÁR, J. Amer. Chem. Soc. *65*, 1522 (1943).

[2] L. ZECHMEISTER, A. L. LEROSEN, W. A. SCHROEDER, A. POLGÁR, and L. PAULING, J. Amer. Chem. Soc. *65*, 1940 (1943).

[1] F. J. PETRACEK and L. ZECHMEISTER, J. Amer. Chem. Soc. *74*, 184 (1952). – B. K. KOE and L. ZECHMEISTER, Arch. Biochem. Biophys. *46*, 100 (1953).

[2] In collaboration with J. H. PINCKARD (unpublished).

or cinnamalazine[1] (Figs. 12–14). There is, however, one exception in the carotenoid series[2]: No clear *cis*-peak phenomenon appeared in the set of dehydro-*β*-carotene (IV) and a few similar compounds with "retro" structure[3], i.e. containing a single bond in the center but double bonds between the hydroaromatic rings and the main aliphatic chain. A theoretical interpretation of this "anormal" behavior is not available at the present time.

### *Steric Configuration and Infrared Spectra*

Considering the nature of the vibrations responsible for the spectral bands observed in the infrared region, it was expected that the shape of the infrared curve would also depend on the spatial configuration of a polyene. Recently, Mag. KAARE LUNDE and the present writer have carried out pertinent studies, as yet unpublished, whose results can be summarized as follows.

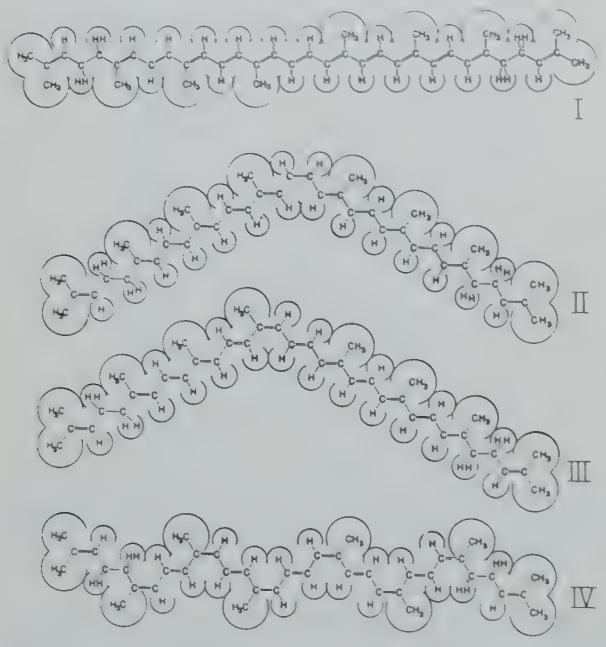



Fig. 11.–Molecular models of some stereoisomeric lycopenes: I, all-*trans*; II, central mono-*cis*; III, another mono-*cis*; and IV, a poly-*cis* form. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

Diphenylbutadiene may assume three configurations, viz. *trans-trans*, *cis-trans*, and *cis-cis*. The two *cis* isomers show in carbon tetrachloride solution around 7·25 $\mu$ a band that does not appear in the *trans-trans* curve. A similar statement is valid for the region, 12·5 $\mu$ – 15 $\mu$ (two "*cis*-peaks"; cyclohexane solution). On the other hand, between 10·0 $\mu$ and 10·6 $\mu$, a typical "*trans*-peak" is observed that decreases and then disappears during the stepwise rearrangement, *trans-trans* → *cis-trans* → *cis-cis*.

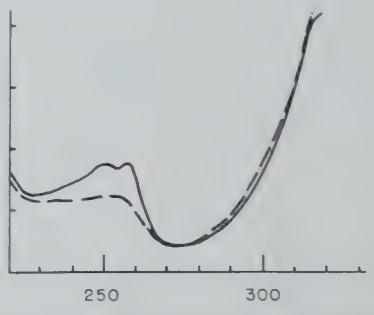



Fig. 12.–*Cis*-peak effect in the phytofluene set: Extinction curves, in hexane: ———, a *cis* form occuring in tomatoes, before iodine catalysis; and – – –, after iodine catalysis. [From: J. Amer. Chem. Soc. *74*, 184 (1952).]

The results obtained when some other representatives of the diphenylpolyene series were used are consistent with those just mentioned.

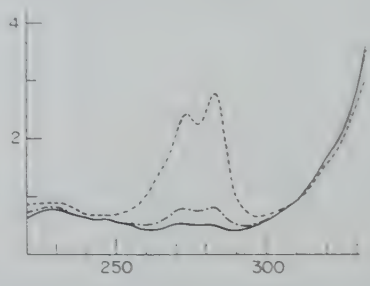



Fig. 13.–*Cis*-peak effect in the diphenyloctatetraene set, in hexane: ———, all-*trans* form; – – – –, a *cis* form; and ·–·–·, curve after iodine catalysis (unpublished).

In the field of the carotenoids several *cis* forms of α-carotene, *β*-carotene, *γ*-carotene, lycopene, and certain hydroxylated compounds have been tested. We are obliged to Professor H. H. INHOFFEN of the Technische Hochschule in Braunschweig for a sample of his

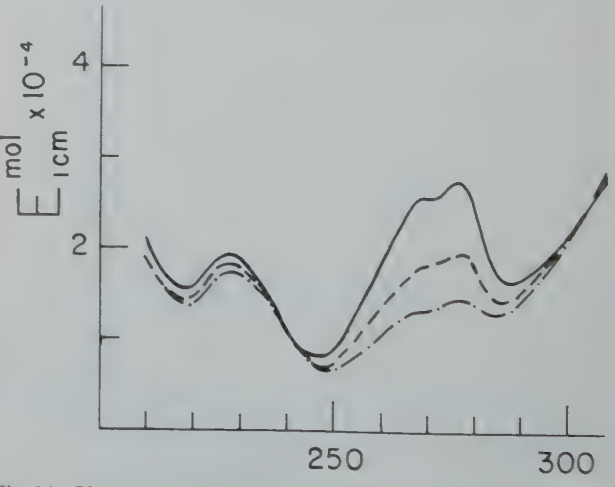



Fig. 14.–*Cis*-peak effect in the cinnamalazine set, in hexane: Extinction curves of *cis*-I cinnamalazine: ———, fresh solution; – – – –, after 150 min illumination with a daylight lamp; and ·–·–·, after exposure to sunshine. [From: J. Amer. Chem. Soc. *75*, 2379 (1953).]

[1] J. DALE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 2379 (1953).

[2] L. ZECHMEISTER and L. WALLCAVE, J. Amer. Chem. Soc. *75*, 5341 (1953).

[3] Cf. W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295 (1952).

synthetic central mono*cis*-$\beta$-carotene[1]. This well-crystallized compound of known configuration shows a strong absorption maximum at 12·84 $\mu$ (Fig. 15) that is missing in the corresponding all-*trans* curve and, indeed, could not be observed in any other all-*trans* spectrum so far.

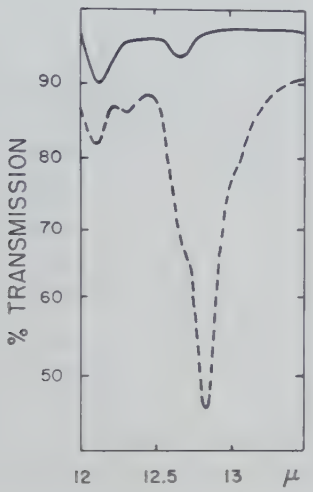



Fig. 15.–Infrared spectral curves in cyclohexane (1 mm NaCl cell): ———, 0·50% solution of all-*trans*-$\beta$-carotene; and – – – –, 1·0% solution of synthetic central mono*cis*-$\beta$-carotene (unpublished).

The 7·25 $\mu$ band included in the two *cis*-diphenylbutadiene curves is also present, with variable intensity, in the spectra of all *cis* carotenoids tested so far and belonging to various stereoisomeric sets. There is, however, one exception: This maximum is missing in the spectrum of central mono*cis*-$\beta$-carotene whose molecular symmetry is comparable to that of the corresponding all-*trans* compound.

None of the all-*trans* pigments studied showed maxima either in the 7·25 $\mu$ or in the 13 $\mu$ region. A 13 $\mu$ band did appear in the spectra of some *cis* carotenoids.

It can be predicted with safety that comparative readings taken with the infrared spectrophotometer will become increasingly useful tools in the stereochemical exploration of polyenes. Infrared spectra may extend and complete the information gained in the visible and ultraviolet regions.

### *Biological Significance of cis-trans Isomerism in $C_{20}$-Systems*

The dependence of the properties of stereoisomeric polyenes on the molecular shape is, as we have seen, so sharp that spatial configurations, especially of vitamins A and of some carotenoids, were expected to influence certain biological processes.

The vitamin A molecule (I) contains four aliphatic double bonds and should occur in 16 spatial forms, four of which are unhindered. "Ordinary" vitamin A has the all-*trans* configuration; however, according to ROBESON and BAXTER[1], one third of many fish liver oils consists of a crystallizable $\Delta^5$-*cis* isomer termed neovitamin A. In some red tuna oils more than half of the vitamin was found in the *cis* configuration[2]. Likewise, synthetic A-concentrates contain substantial amounts of the neovitamin. Evidently, a catalytic interconversion, *trans* vitamin $\rightleftarrows$ *cis*-vitamin(s) takes place both *in vitro* and *in vivo*. Physiologically speaking, what we term vitamin A is a mixture of *cis-trans* isomers[3] and displays a weaker action in the body than would the pure all-*trans* compound. The biopotency of neovitamin A amounts to 70–80 % of the *trans* form[4].

Both structurally and biologically closely related to vitamin A is retinene which, according to MORTON[5], is the aldehyde (II) corresponding to vitamin A. This compound plays a key role in the chemistry of the "visual cycle" which has been the object of successful investigations carried out by WALD and his school and extending over two decades[6]. According to WALD, vertebrate rod vision involves the cleavage and resynthesis of the "visual purple", termed rhodopsin, a polyene-proteid which under the influence of light dissociates into retinene and the protein opsin. In the retina most of the retinene is converted into vitamin A from which rhodopsin is built up again, thus closing the cycle.

Recently, rhodopsin has been synthesized *in vitro* by HUBBARD and WALD[7], who made use of a vitamin A concentrate, opsin, liver alcohol dehydrogenase, and cozymase. When the vitamin concentrate was replaced by pure crystalline all-*trans*-vitamin A, surprisingly enough, no rhodopsin was formed. However, when the authors converted the *trans* vitamin, by means of iodine catalysis, into a mixture of stereoisomers, the mixture did effect the rhodopsin synthesis[8]. Evidently, this vitally important biological process requires a *cis* configuration of the polyene component.

After having prepared *in vitro* five crystalline stereoisomeric *cis* retinenes, the same authors have

[1] H. H. INHOFFEN, F. BOHLMANN, K. BARTRAM, G. RUMMERT, H. POMMER, F. WESTPHAL, and G. LINHOFF, Liebigs Ann. Chem. *570*, 54 (1950).

[1] CH. D. ROBESON and J. G. BAXTER, J. Amer. Chem. Soc. *69*, 136 (1947). For a synthetic 3-*cis*-vitamin A, cf. W. GRAHAM, D. A. VAN DORP and J. F. ARENS, Rec. Trav. chim. Pay-Bas *68*, 609 (1949). Survey: J. G. BAXTER, Fortschr. Chem. organ. Naturstoffe *9*, 41 (1952).

[2] P. MEUNIER and J. JOUANNETEAU, Bull. Soc. chim. biol. *30*, 260 (1948).

[3] J. D. CAWLEY, CH. D. ROBESON, L. WEISLER, E. M. SHANTZ, N. D. EMBREE, and J. G. BAXTER, Science *107*, 346 (1948).

[4] P. L. HARRIS, S. R. AMES, and J. H. BRINKMANN, J. Amer. Chem. Soc. *73*, 1252 (1951).

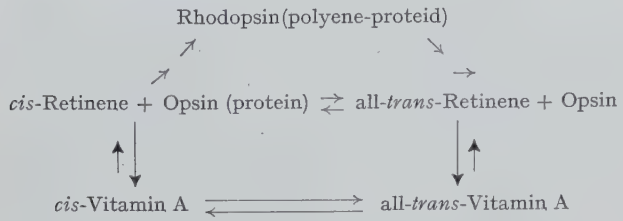
[5] R. A. MORTON, Nature (London) *153*, 69 (1944).

[6] General Survey: G. WALD, *The Chemical Evolution of Vision*, The Harvey Lectures *41*, 117 (1945–1946).

[7] R. HUBBARD and G. WALD, Proc. Nat. Acad. Sci. (U.S.A.) *37*, 69 (1951). – G. WALD, Science *113*, 287 (1951).

[8] R. HUBBARD and G. WALD, Science *18*, 60 (1952); J. gen. Physiol. *36*, 269 (1952–1953).

demonstrated that the particular spatial form liberated from rhodopsin during the cycle has a molecular shape different from that which is required for building up rhodopsin. "The stereoisomerization of retinene or of the corresponding vitamin A is an integral and necessary component of the rhodopsin cycle." Hence, it must constitute a normal function of the body. Very probably, the active isomer is supplied to the eye continuously by the blood stream. The simplified outlines of the visual cycle taking place in the retina are formulated in *Scheme 1*.




Scheme 1. Visual cycle.

### *Biological Significance of cis-trans-Isomerism in $C_{40}$-Systems*

Our present knowledge that the carotenes represent provitamins A and are (in part) converted in the mammal body into the vitamin by a split in the center of their molecules[1],

$$C_{40}H_{56} \rightarrow 2\,C_{20}H_{29} \cdot OH$$

can be traced back to some basic observations made by Steenbock *et al.*[2] and by Moore[3].

Much later, when the phenomena of polyene stereoisomerization were detected, the question arose how far the biopotencies of the four most common naturally occurring provitamins A, viz. α-carotene, β-carotene, γ-carotene, $C_{40}H_{56}$, and cryptoxanthin, $C_{40}H_{55}$·OH, can be influenced by bending their straight all-*trans* molecules. For the contributions of several authors to this field we may refer to the literature[4].

Systematic investigations into the problem mentioned have been carried out in close collaboration with Dr. H. J. Deuel, Jr. of the University of Southern California and his group[5]; and recently, the results were discussed in two survey articles[6].

[1] P. Karrer, R. Morf, and K. Schöpp, Helv. chim. Acta *14*, 1036, 1431 (1931).

[2] H. Steenbock, M. T. Sell, E. M. Nelson, and M. V. Buell, J. Biol. Chem. *46*, Proc. XXXII (1921).

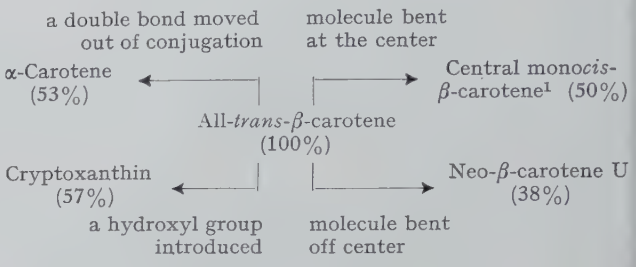
[3] Th. Moore, Lancet *217*, 380 (1929); Biochem. J. *23*, 803, 1267 (1929).

[4] A. E. Gillam and M. S. El Ridi, Biochem. J. *30*, 1735 (1936); the same authors and K. Kon, Biochem. J. *31*, 1605 (1937). Later contributions: A. R. Kemmerer and G. S. Fraps, J. Biol. Chem. *161*, 305 (1945); Ind. Eng. Chem. anal. Ed. *15*, 714 (1943). – B. W. Beadle and F. P. Zscheile, J. Biol. Chem. *144*, 21 (1942).

[5] Arch. Biochem. Biophys. *5* (1944). – *40* (1952); recent. communication: H. J. Deuel, Jr., H. H. Inhoffen, J. Ganguly, L. Wallcave, and L. Zechmeister, Arch. Biochem. Biophys. *40*, 352 (1952).

[6] L. Zechmeister, *Stereoisomeric Provitamins A*. Vitamins and Hormones *7*, 57 (1949); *Some Biochemical Studies Based on Chromatographic Methods*. The Harvey Lectures *47*, 221 (1951–1952).

As is well known, the bioeffect of a given provitamin A can be determined quantitatively on the basis of the total gain-in-weight of rats or chicks receiving, after a depletion period, a certain daily dose (micrograms) of the carotenoid tested. Since all-*trans*-β-carotene induces the highest weight increase, its effect is conveniently set equal to 100%. This potency can be substantially decreased either by some structural or by stereochemical variations in the molecules. Significantly, both types of alterations result in a biological weakening of the same order of magnitude as shown




Scheme 2. Weakening of the bioeffect of all-*trans*-β-cartotene by some chemical reactions and spatial rearrangements.

in *Scheme 2* (the figures represent relative provitamin A potencies).

The figures given in Scheme 2 are, evidently, resultants of many partial bio-processes. Among other factors, they seem to have been influenced by the extent to which a certain molecular shape may be fitted into an enzyme system. Of course, it would be temptingly simple to assume that in the body each *cis* provitamin must first be stereoisomerized to the corresponding all-*trans* form, before it can be subjected to the enzymatic attack. However it has been found recently that a naturally occurring *cis* pigment termed pro-γ-carotene shows a bio-potency equal to, or even stronger than, that of all-*trans*-γ-carotene[2]. Consequently, it seems improbable that the numerous *cis* provitamins known should all be inactive *per se*, and that absolute stereochemical bio-specificity would prevail in the enzymatic cleavage system in favor of the all-*trans* configuration.

### *Conclusion*

It was attempted to demonstrate to the non-specialist reader that the class of open-chain polyenes constitutes an exceptionally good example of the strong influence of the molecular shape on the characteristics of a compound. The richness in steric possibilities, unique in the realm of low-molecular substances, offers various points of attack to the investigator but also involves considerable difficulties. These can be,

[1] L. Zechmeister, H. J. Deuel, Jr., H. H. Inhoffen, J. Leemann, S. M. Greenberg, and J. Ganguly, Arch. Biochem. Biophys. *36*, 80 (1952).

[2] S. M. Greenberg, C. E. Calbert, J. H. Pinckard, H. J. Deuel, Jr., and L. Zechmeister, Arch. Biochem. Biophys. *24*, 31 (1949).

in part, overcome by the combination of such different methods as selective adsorption, iodine catalysis, spectroscopy, total synthesis, and bioassays. It is hoped that the field under review may attract the interest of some physicists, chemists and biologists.

Perhaps this writer may close the present incomplete survey of a limited field with a quotation referring to VAN T'HOFF's monumental work that has laid the foundation to all subsequent stereochemical studies: «... je crois qu'il y a là une voie nouvelle dans laquelle il est bon de s'engager, avec prudence sans doute, mais avec une persévérance que les résultats déjà entrevus paraissent justifier[1].»

*Zusammenfassung*

Polyene, die ein konjugiertes Doppelbindungssystem in offener Kette enthalten, können zahlreiche raumisomere Formen annehmen. In den meisten zu dieser Klasse gehörenden Naturstoffen sowie in Produkten der Synthese wiegt das geradlinige «all-*trans*»-Isomer vor, das aber durch thermische oder photochemische Eingriffe oder durch Jodkatalyse in ein kompliziertes Gemisch von *cis-trans*-Formen umgelagert werden kann. Derartige Gemische lassen sich chromatographisch in ihre Bestandteile zerlegen. Diese Beobachtungen gelten sowohl für isoprenoide Verbindungstypen (carotinoide Farbstoffe, Phytofluen, Vitamin A, Retinen) als auch für Systeme mit unverzweigtem Kohlenstoffgerüst (Diphenylpolyene, Azine).

Die *trans* → *cis*-Umlagerung hat in vielen Fällen eine räumliche Hinderung zu überwinden, deren Grundtypen besprochen werden.

Durch Änderung der stereochemischen Konfiguration eines Polyens werden seine spektroskopischen Merkmale tiefgehend beeinflusst, und zwar im sichtbaren, im ultravioletten und im infraroten Wellenlängengebiet. Der «*cis*-peak-Effekt» dient als ein Mass der Biegung der Moleküle.

Auch in gewissen biologischen Vorgängen spielt *cis-trans*-Isomerie eine wichtige Rolle; so ist zum Beispiel für den Sehakt ein bestimmter stereo-spezifischer Teilvorgang eine Voraussetzung. Ferner büssen Provitamine A einen grossen Teil ihrer Aktivität im Organismus ein, wenn ihre *trans*-Moleküle morphologische Variationen erleiden. Auch hier zeigt sich ein enger Zusammenhang zwischen Biopotenz und Molekularform.

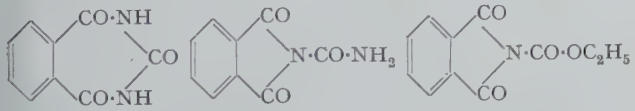
[1] From a letter sent by WURTZ to VAN T'HOFF in 1877; cf. E. COHEN, *Jacobus Henricus van t'Hoff* (Akad. Verlagsges., Leipzig 1912).

# Brèves communications - Kurze Mitteilungen
# Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. – Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed by their correspondents.

## Die Konstitution des Phthalylharnstoffs

Phthalylharnstoff, der durch Einwirkung von Phosphoroxychlorid auf Phthalursäure[1] oder von Phthalylchlorid auf Harnstoff[2] gewonnen werden kann, wurde bisher immer als I formuliert, obwohl seine Herstellung, Eigenschaften und Reaktionen auch mit Formel II in bestem Einklang stehen.




I II III

Wir konnten im Laufe unserer Versuche eine Beobachtung machen, die für Formel II sprach. Als sich nämlich das von HELLER und JACOBSOHN[3] beschriebene Phthalylurethan (III) gegenüber Ammoniak als sehr reaktionsfähig erwies, indem es in alkoholischer Lösung mit überschüssigem konzentriertem wässerigem Ammoniak schon bei Zimmertemperatur unter Ringöffnung *Phthalamid* gab, prüften wir auch Phthalylharnstoff auf sein Verhalten gegenüber Ammoniak unter den gleichen Bedingungen. Es ergab sich, dass Phthalylharnstoff, ganz gleich dem Phthalylurethan, mit Ammoniak unter Bildung von Phthalamid reagierte. Auch mit Hydrazinhydrat reagierten beide Verbindungen ganz analog unter Bildung von *Phthalhydrazid*.

Einen Beweis für die Richtigkeit der Formel II lieferte uns schliesslich das Ergebnis der Umsetzung von Phthalylharnstoff mit Xanthydrol. Bekanntlich setzen sich monosubstituierte und asymmetrisch disubstituierte Harnstoffe mit Xanthydrol in Essigsäure unter Bildung von Monoxanthylderivaten um[1], während zyklische Ureide, wie Parabansäure[2] und 5, 5-disubstituierte Barbitursäuren[3], Dixanthylderivate liefern. Phthalylharnstoff

[1] A. PIUTTI, Liebigs Ann. Chem. *214*, 17 (1882). – C. S. SMITH und C. J. CAVALLITO, J. Amer. Chem. Soc. *61*, 2218 (1939).

[2] T. W. EVANS und W. M. DEHN, J. Amer. Chem. Soc. *51*, 3651 (1929).

[3] G. HELLER und P. JACOBSOHN, Ber. dtsch. chem. Ges. *54*, 1107 (1921).

[1] R. FOSSE, C. r. Acad. Sci. *145*, 813 (1907); *158*, 1432 (1914). – W. ADRIANI, Rec. Trav. chim. *35*, 180 (1915).

[2] Vgl. R. FOSSE, *L'Urée* (Presses Universitaires de France, Paris 1928), S. 261.

[3] R. FABRE, J. Pharm. Chim. [7] *26*, 241 (1922); Bull. Soc. chim. France [4] *33*, 791 (1923).

gab mit Xanthydrol in Essigsäure ausschliesslich ein *Monoxanthylderivat*, das, aus einem Gemisch von Dioxan und Alkohol umkristallisiert, in farblosen Nadeln vom Smp. 201–202° (ber. N 7,56%; gef. N 7,37%) erhalten wurde, wobei es ohne Einfluss war, ob 1 oder 2 Mol Xanthydrol zur Anwendung kamen.

Die so auf chemischem Wege erwiesene Konstitutionsformel II des Phthalylharnstoffs wurde auch durch röntgenographische Untersuchungen von Grdenić und Bezjak[1] bestätigt.

V. Hahn, P. Hammes und Z. Gerić

*Institut für organische Chemie der Technischen Fakultät, Universität Zagreb, den 5. August 1953.*

*Summary*

On the basis of experimental evidence, it is proved that phthalyl urea has the structure II, and not structure I, proposed by Piutti and generally accepted by other investigators.

[1] Arhiv kem. (im Druck).

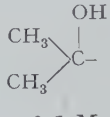
## Über die Konstitution des Visamminols

Aus den Samen von *Ammi visnaga* L. (Umbelliferae), die das koronardilatorisch wirksame Khellin enthalten, haben kürzlich Smith, Pucci und Bywater[1] neben kristallisiertem Visnagan einen weiteren kristallinen Inhaltsstoff (RI-788) vom Smp. 157–159° und der spez. Drehung $[\alpha]_D = +96°$ isoliert. Der neue Naturstoff der Formel $C_{15}H_{16}O_5$ (MG. gef. 276) gibt ein gelbes Hydrochlorid. Sein UV.-Spektrum ist demjenigen von Dihydrokhellin[2] sehr ähnlich; in der koronardilatorischen Wirksamkeit entspricht er dem Khellin.

Dieselbe, aber noch unreine Substanz (Smp. 133–140°) hatten offenbar schon früher Cavallito und Rockwell[3] in Händen. Weitere Untersuchungen über diese, vermutlich ein Chromon darstellende Verbindung liegen nicht vor.

Inzwischen haben auch wir aus den Samen von *Ammi visnaga* durch Chromatographie eine kleine Menge (0,5 g) dieses Stoffes isoliert.

Der farblose Pflanzenstoff, für den wir den Namen Visamminol (I) vorschlagen möchten, schmilzt bei 160–160,5° und besitzt in Übereinstimmung mit den amerikanischen Autoren ein $[\alpha]_D = +93 \pm 3°$ (Chloroform) und die Bruttoformel $C_{15}H_{16}O_5$. Anwesend sind zwei Hydroxylgruppen (Zerewitinoff) und zwei $CH_3(C)$-Gruppierungen (Kuhn-Roth). Methoxylgruppen (Zeisel) fehlen. Die weinrote Eisen(III)-chlorid-Reaktion spricht für das Vorliegen einer 5-ständigen Hydroxylgruppe[4], die blaue Gibbssche Reaktion für ein in p-Stellung unsubstituiertes Phenol. Mit Essigsäureanhydrid-Natriumazetat entstand das Diazetat II [$C_{19}H_{20}O_7$; Smp. 147,5–148,5°; kein aktives H; keine Eisen(III)-chlorid-Reaktion]. Erhitzen mit Dimethylsulfat-Kaliumkarbonat in Azeton verwandelte I in den Monomethyläther III $C_{15}H_{15}O_4 \cdot OCH_3$ vom Smp. 142–142,5°. III gab keine Eisen(III)-chlorid-Farbreaktion, keinen Gibbsschen Test, aber – im Gegensatz zu I – eine rotorange Farbe mit Kalilauge in Cellosolve. Letztere Reaktion deutet auf ein 2-Methylchromon hin[1]. Die nichtphenolische Hydroxylgruppe liegt sehr wahrscheinlich in der Gruppierung



vor, da mit Chromsäure 0,6 Mol Azeton (als p-Nitrophenylhydrazon identifiziert) entstanden. Mit wasserfreier p-Toluolsulfonsäure in siedendem Benzol entstand aus Visamminol (I) in guter Ausbeute die optisch inaktive, gelbgefärbte Verbindung IV, Smp. 120–121°; $C_{15}H_{14}O_4$. Positive Gibbssche Farbreaktion. Die Eisen(III)-chlorid-Reaktion war jetzt, ähnlich wie beim 2-Methyl-5-oxy-4′,5′-6,7-furano-chromon (VII)[2], (blau-)grün. IV lieferte mit Methyljodid und Kaliumkarbonat in siedendem Azeton den Methyläther V [$C_{15}H_{13}O_3 \cdot OCH_3$; Smp. 108–109°; keine Eisen(III)-chlorid-Reaktion, rote KOH-Reaktion]. Beim Erhitzen von IV mit 2–8%iger wässriger Kalilauge im Stickstoffstrom resultierten 0,75 Mole Azeton (p-Nitrophenylhydrazon) und der Stoff VI $C_{11}H_{12}O_3$ vom Smp. 175°. [Keine Eisen(III)-chlorid-Reaktion; blauviolette Gibbssche Reaktion.] VI besitzt ein UV-Spektrum ($\lambda_{max} = 256$ m$\mu$ [$\lg \varepsilon = 4{,}2$] $\lambda_{min} = 233$ m$\mu$ [$\lg \varepsilon = 3{,}78$], Inflexion etwa 280 m$\mu$ [$\lg \varepsilon = 3{,}25$] Alkohol), das demjenigen von 4,6-Dimethoxykumaron[3] sehr ähnlich ist. Milde NaOH-KOH-Schmelze von I lieferte Phloroglucin (als Triazetat identifiziert). Ozonolyse einer sehr kleinen Menge von IV liess Essigsäure und Isobuttersäure, Ozonolyse von wenig VI liess Isobuttersäure und Phloroglucinaldehyd entstehen. Der geringen Menge wegen mussten die letzten drei Abbauprodukte papierchromatographisch identifiziert werden.

[Essigsäure $RF = 0{,}18$; Isobuttersäure $RF = 0{,}35$, gef. $RF = 0{,}18$; 0,35; Lösungsmittel: abs. Alkohol: Wasser: konz. Ammoniak = 95:5:1. Nachweis mit Umbelliferon-UV.-Licht. Phloroglucin-aldehyd $RF = 0{,}40$ bzw. 0,63 gef. $RF = 0{,}39$–0,40 bzw. 0,63 (neben anderen schwächeren Flecken); Lösungsmittel kohlensäuregesättigtes Wasser bzw. Isopropanol: Wasser = 1:4. Nachweis mit Eisen(III)-chlorid und diazotiertem Benzidin. Die Chromatogramme wurden aufsteigend auf Whatmanpapier Nr. 1 ausgeführt.]

Die Versuche zeigen, dass Visamminol (I) ein 2-Methyl-5,7-dioxychromon-Derivat darstellt und dass das Abbauprodukt VI die Formel VI besitzt. In IV liegt ein 2′-Isopropylfurano-chromon vor, während I einen Dihydrofuranring enthalten muss, da im Spektrum von I die langwellige Bande um 46 m$\mu$ hypsochrom gegenüber derselben Bande von IV verschoben ist. Ein 2′-Oxy- oder ein 3′-Oxyfuranring scheidet aus, weil solche Stoffe schon bei sehr milden Bedingungen in Furane übergehen müssten[4] und mit Chromsäure kein oder nur sehr wenig Azeton geben würden. Ausser Betracht fallen auch 3′- oder 4′-Oxy-2′,2′-dimethylchromanringe. I enthält daher eine 2′-tert.-Oxy-isopropyl-2′,3′-dihydrofuran-Gruppierung, die bei der Wasserabspaltung unter Allylumlagerung in einen Furanring übergeht. Über die Verknüpfungsstellen dieses Ringes mit dem Chromonskelett geben die UV.-Spektren Aufschluss: Schon die Ähnlichkeit der

[1] E. Smith, L. A. Pucci und W. C. Bywater, Science *115*, 520 (1952).

[2] J. S. H. Davies und W. L. Norris, J. chem. Soc. *1950*, 3195.

[3] C. J. Cavallito und H. E. Rockwell, J. org. chem. *15*, 820 (1950).

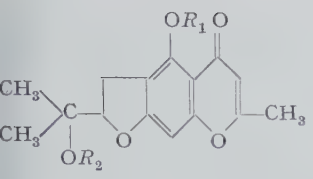
[4] A. Bolleter, K. Eiter und H. Schmid, Helv. chim. Acta *34*, 186 (1951).

[1] A. Schönberg und A. Sina, Amer. Soc. *72*, 1611 (1950); J. chem. Soc. *1950*, 3344.

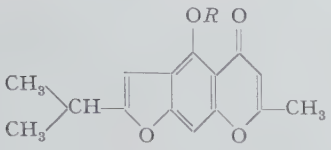
[2] A. Schönberg und N. Badran, Amer. Soc. *73*, 2960 (1951).

[3] H. Bickel und H. Schmid, Helv. chim. Acta *36*, 671 (1953).

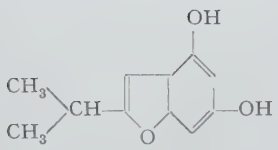
[4] 3-Oxycumaran spaltet schon beim Erhitzen in wässriger Lösung Wasser ab. [R. Stoermer und W. Koenig, Ber. dtsch. chem. Ges. *39*, 499 (1906)]; dasselbe würde für ein 2-Oxycumaran gelten, wenn es als Halbketal überhaupt beständig wäre.



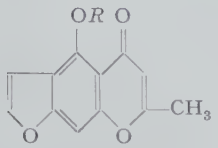
I: $R_1 = R_2 = H$
II: $R_1 = R_2 = COCH_3$
III: $R_1 = CH_3$; $R_2 = H$



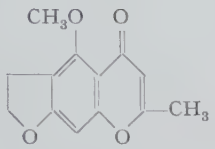
IV: $R = H$
V: $R = CH_3$



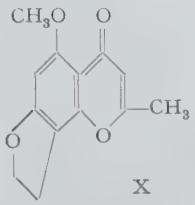
VI



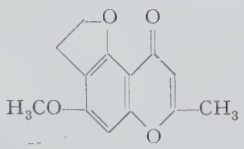
VII: $R = H$
VIII: $R = CH_3$



IX



X



XI

Spektren von Visamminol (I) und dem 2,6-Dimethyl-5-oxy-7-methoxychromon (Eugenitin) bzw. deren Azetate (Abb. 1) weisen auf ein 6-alkyliertes Chromon hin[1]. Die Spektren von Visamminol-methyläther (III) und Dihydrovisnagin (IX) sind fast gleich; deutlich verschieden sind sie von den Kurven des isomeren allo-Dihydrovisnagins (X) und des Iso-dihydrovisnagins (XI)[2] (Abb. 1). Ferner sind die

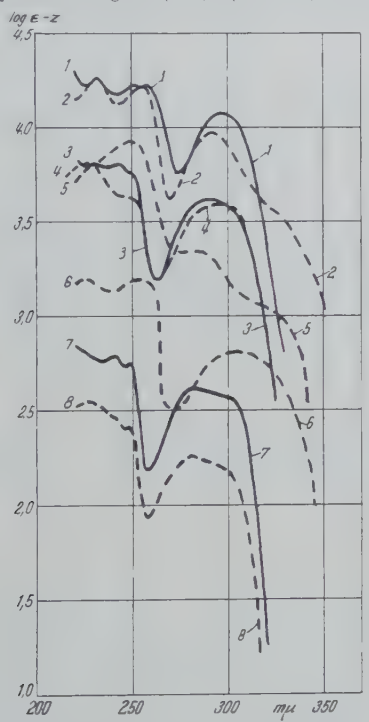



Abb. 1. *UV.-Spektren* (in Alkohol). Kurve 1, Visamminol (I) ($Z = 0$); Kurve 2, Eugenitin ($Z = 0$)[3]; Kurve 3, Visamminol-methyläther (III) ($Z = 0{,}5$); Kurve 4, Dihydro-visnagin (IX) ($Z = 0{,}5$)[4]; Kurve 5, Dihydro-isovisnagin (XI) ($Z = 0{,}5$)[4]; Kurve 6, Dihydro-allovisnagin (X) ($Z = 1{,}0$)[4]; Kurve 7, Visamminol-diazetat (II) ($Z = 1{,}5$); Kurve 8, Eugenitin-azetat ($Z = 1{,}8$).

Spektren von IV bzw. V mit Ausnahme der durch den Isopropylrest verursachten Rotverschiebung von 4–9 mμ praktisch identisch mit den Kurven von VII bzw. Visnagin (VIII).

Die Formel I, die wir deshalb dem Visamminol zuteilen, zeigt erneut die nahe biogenetische Verwandtschaft von natürlichen Chromonen und natürlichen Kumarinen auf. Der 2′-tert.-Oxyisopropyl-2′,3′-dihydrofuran-Rest findet sich nämlich auch im Kumarin (+)-Marmesin[1] (aus *Aegle marmelos*, Correâ [Rutaceaє]) und in seinem Antipoden, dem (–)Nodakenetin aus dem Glukosid Nodakenin[2] (aus *Peucedanum decursivum* Maxim. [Umbelliferae]).

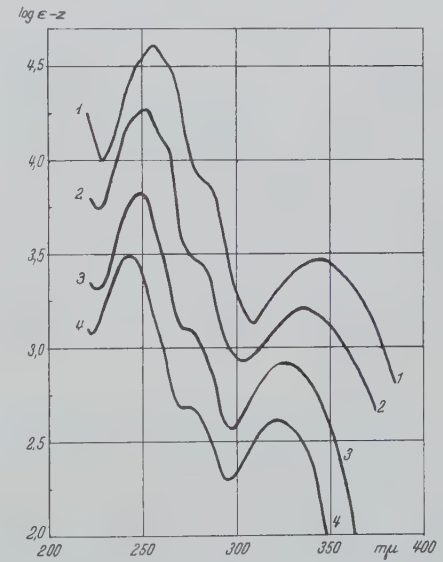



Abb. 2. *UV.-Spektren* (in Alkohol). Kurve 1, Verbindung IV ($Z = 0$); Kurve 2, Verbindung VII ($Z = 0{,}3$); Kurve 3, Verbindung V ($Z = 0{,}8$); Kurve 4, Visnagin (VIII) ($Z = 1{,}1$).

Eine ausführliche Mitteilung über den Gegenstand wird später an anderer Stelle veröffentlicht werden.

[1] Bezüglich des Unterschiedes der Spektren von 6 und 8 alkylierten 5,7-Dioxychromonen siehe A. Bolleter, K. Eiter und H. Schmid, Helv. chim. Acta *34*, 186 (1951).

[2] Eine XI entsprechende Formel für Visamminol scheidet schon wegen seiner Schwerlöslichkeit in verdünnter Lauge und der positiven Eisen(III)-chlorid-Reaktion aus.

[3] Vgl. H. Schmid, Helv. chim. Acta *32*, 813 (1949).

[4] Diese Spektren haben wir der Arbeit von J. S. H. Davies und W. L. Norris, Soc. *1950*, 3195, entnommen.

[1] A. Chatterjee und S. S. Mitra, Amer. Soc. *71*, 606 (1949).

[2] J. Arima, J. chem. Soc. Japan *48*, 88, 457 (1927); Bull. chem. Soc. Japan *4*, 16, 113 (1929). – E. Späth und P. Kainrath, Ber. dtsch. chem. Ges. *69*, 2062 (1936). – E. Späth und E. Tiray, Ber. dtsch. chem. Ges. *72*, 2089 (1939).

Dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung, Bern, danken wir bestens für die gewährte Hilfe.

W. Bencze und H. Schmid

*Chemisches Institut der Universität Zürich, den 12. August 1953.*

*Summary*

The structure of Visamminol (I), a compound isolated from *Ammi visnaga* by Smith, Pucci, and Bywater, has been elucidated by degradation and by a comparison of the UV.-spectra of I and its derivatives with those of other chromones.

## The Effect of Hyperconjugation upon the Boiling Points of Alkenes

The boiling point is conceived as the perceptible manifestation of the underlying physical phenomenon of inter-molecular attraction, called cohesion (Kohäsion) by Hückel[1]. As such it is a function of the degree of electrical asymmetry of the molecule. This asymmetry of charge distribution is known to be the sum of two effects: the permanent or orientation polarization ($P\mu$), and the induced polarization ($P_D$) or "polarizability".

By applying these principles, Hückel has been successful in formulating qualitative correlations between the structures and boiling points of organic compounds, now well-known as "Hückel's boiling-point rules". There remain, however, some cases in which these rules, in their present form, fail to rationalize the factual evidence. Thus Hückel recognized an increase in boiling point with increasing proximity of an alkyl group to a carbon-carbon double bond. Generally, the boiling point *decreases* with increasing proximity of an alkyl group to a polar (e.g. carbonyl) function, and this so-called "shielding effect" is explained by steric inhibition of polarizability by the bulky and only weakly polar alkyl radical. Hückel was unable to explain why this principle seemed to fail in the case where the polar group is a point of carbon-carbon unsaturation. Similarly, irregularities have been noted[2] in that the normal 1-olefins which would be expected to be the highest boiling by virtue of their extreme asymmetry (maximum $P\mu$), actually have lower boiling points than any of their C=C position isomers. There is no rational interpretation of this behavior at the present time.

Now it is clear that the structures of two or more aliphatic or alicyclic mono-olefins differing structurally only in the position of the carbon-carbon double bond (but not in their carbon skeleton) can differ only in their electron distribution, and that the latter must manifest itself in one of the following ways:

(1) in the permanent polarization, $P\mu$

(2) in the induced polarization, $P_D$, by means of either

(I) shielding effects of bulky groups preventing small inter-molecular distances and thus decreasing the effective polarizability, or

(II) intrinsic differences in the degree of electron delocalization which could, depending on their nature, decrease or increase effective polarizability.

As (1) and (I) do not account for the observed facts but are actually in contradiction to them, only (II) can provide a clue. Reflection will show that there must indeed be a difference in electron delocalization of C=C isomeric olefins inasmuch as they differ in the amount of hyperconjugation possible in the respective structures.

To test the resulting hypothesis, namely that hyperconjugation will have a measurable effect upon the boiling point of unsaturated hydrocarbons, a statistical analysis of some hundred compounds was undertaken. The compounds chosen comprise all the aliphatic mono-olefins from $C_4$ to $C_7$, inclusive, which are capable of C=C position isomerism; the n-octenes and methylheptenes; and the normal alkynes from $C_4$ to $C_6$, inclusive. The only principle governing this selection was easy accessibility of boiling point data, which were obtained from the best current sources[1]. The number of hydrogen atoms in α-position with respect to the double bond served as a convenient quantitative means of expressing the "amount" of hyperconjugation. A representative sample will illustrate the methods used (Table).

[1] W. Hückel, *Theoretische Grundlagen der Organischen Chemie* (Akademische Verlags-GmbH., Leipzig, 1935), 2nd ed., 2nd vol., p. 122.

[2] J. A. Leermakers and A. Weissberger in H. Gilman, *Organic Chemistry* (Wiley, New York, N.Y., 1943), p. 1723.

[1] F. D. Rossini *et al.*, *Selected Values of Properties of Hydrocarbons* (National Bureau of Standards, Circular C 461, U. S. Government Printing Office, Washington, D.C., 1947). – G. Egloff, *Physical Constants of Hydrocarbons* (Reinhold Publishing Corp., New York, N. Y., 1953, Volume V).

| Compound | Structure | Hyperconjugation | Boiling Point at 760 mm |
|---|---|---|---|
| 5-Methyl-1-hexene . . . . | $CH_3-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_2-CH_2-CH=CH_2$ | 2 | 85·650 |
| 2-Methyl-3-hexene . . . . | $CH_3-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH=CH-CH_2-CH_3$ | 3 | 86° |
| 5-Methyl-2-hexene . . . . | $CH_3-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_2-CH=CH-CH_3$ | 5 | *cis:* 91°<br>*trans:* 86° |
| 2-Methyl-1-hexene . . . . | $CH_2=\underset{\displaystyle CH_3}{\underset{\vert}{C}}-CH_2-CH_2-CH_2-CH_3$ | 5 | 91·3° |
| 2-Methyl-2-hexene . . . . | $CH_3-\underset{\displaystyle CH_3}{\underset{\vert}{C}}=CH-CH_2-CH_2-CH_3$ | 8 | 95·41° |

The coefficient of rank correlation was computed for each group of isomers, using SPEARMAN's formula:

$$r = 1 - \frac{6\,\Sigma\,(x-y)^2}{n^3-n}$$

where $r$ is the coefficient of rank correlation, $x$ and $y$ are the rank positions of the two variables, and $n$ is the number of members in each series. For the sample given, $r = 0{\cdot}975$. The sample illustrates various causes of imperfect correlation which are inherent in the nature of the data:

Several structures may have the same amount of hyperconjugation and still differ in their boiling points due to determining factors not considered in the present analysis; and boiling points of structures which give rise to *cis-trans* isomerism are difficult to arrange in order of rank. Occasionally, only the boiling point of steric mixtures is recorded; where boiling points of sterically pure isomers are available, it is impossible to choose either one in fairness as the difference between them is unrelated to hyperconjugation and probably due to a change in $P\mu$.

Nevertheless, the correlations calculated as shown are equal to one in most, and higher than 0·8 in all series studied. The deviations which do exist can invariably be traced to difficulties such as those mentioned above. More important, in spite of these difficulties, the probability of the null hypothesis (i.e. the hypothesis that there is no dependency between hyperconjugation and boiling point in the compounds concerned) can be calculated by standard statistical methods to be in the order of $10^{-25}$, or definitely negligible.

Concluding, a positive effect of hyperconjugation upon the boiling points of aliphatic mono-olefins may be considered established. The magnitude of this effect is such that it completely supersedes that of symmetry of charge distribution in these weakly polar (i.e. essentially quadrupolar) molecules. It is to be expected that analogous relationships can be found in other classes of organic compounds.

C. W. BECK

*Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Mass., U.S.A., August 23, 1953.*

*Zusammenfassung*

Es wird gezeigt, dass die Hyperkonjugation einen entscheidenden Einfluss auf den Siedepunkt der einfach ungesättigten aliphatischen Kohlenwasserstoffe hat. Dieser Zusammenhang wird durch vergrösserte Elektronendelokalisierung in Molekülen, die Hyperkonjugation aufweisen, theoretisch verständlich. Ein grösseres Ausmass der Elektronendelokalisierung führt zu stärkerer Kohäsion und dadurch zu erhöhten Siedepunkten. Die neue Gesetzmässigkeit ergänzt ohne Widerspruch die bekannten Hückelschen Siedepunktsregeln und erklärt Beobachtungen, die aus keiner der bisher aufgestellten Regeln ableitbar waren. Es ist zu erwarten, dass analoge Zusammenhänge in anderen Gruppen von organischen Verbindungen aufgewiesen werden können.

## Zur bakteriziden Wirkung von komprimiertem Sauerstoff

Es ist bekannt, dass Sauerstoff unter Druck das Wachstum aller Mikroorganismen hemmt[1]. Dieser bakteriostatische Effekt wird bei längerer Einwirkungszeit und mit steigender Temperatur bakterizid[2]. Worauf diese keimabtötende Wirkung beruht, ist bisher ungeklärt. Toxische Stoffwechselprodukte können, wenn sie überhaupt auftreten, das Phänomen nicht erklären, da nach Beseitigung des Druckes das Wachstum sofort wieder einsetzt. Es ist weiter bekannt, dass komprimierter Sauerstoff die Atmung und den Stoffwechsel von Mikroorganismen in starkem Masse beeinträchtigt[3]. Daher liegt die Vermutung nahe, dass die Bakterien in ihrer Nährlösung verhungern, weil sie nicht in der Lage sind, die gebotenen Nährstoffe zu verwerten. Um diese Frage zu klären, wird *Escherichia coli* in flüssigem Nährsubstrat gezüchtet, abzentrifugiert, gewaschen und 24 h in physiologischer Kochsalzlösung bebrütet, um vorhandene Reservestoffe zu verbrauchen. Anschliessend setzt man die eine Hälfte der Bakterienaufschwemmung in «Standard-Merck-I-Bouillon» unter 8 atü $O_2$ und belässt den Rest unter gewöhnlichen Bedingungen in der physiologischen NaCl-Lösung. Die Bakterien sterben unter komprimiertem Sauerstoff schneller ab als in der Kochsalzlösung (siehe Abb.). Erhitzt man Coli-Bakterien kurzfristig auf höhere Temperatur, so ist die Absterberate unter 8 atü $O_2$ stets grösser als unter gewöhnlichem Luftdruck (Tab.). Aus diesen Gründen scheint ein Verhungern nicht die Ursache der bakteriziden Wirkung zu sein.

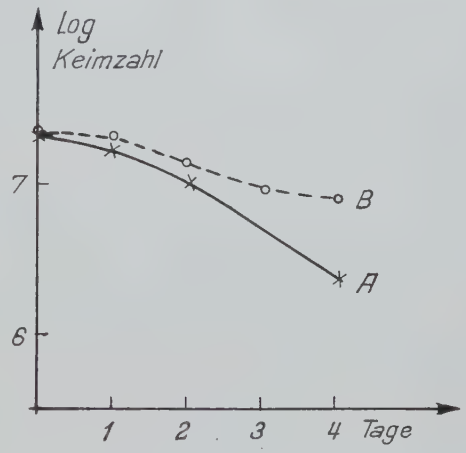



Abb. 1. Abnahme der Keimzahlen von *Escherichia coli* bei 26°C. *A* unter 8 atü $O_2$ in «Merck-I-Bouillon»; *B* unter gewöhnlichem Luftdruck in physiologischer Kochsalzlösung.

Es erhebt sich weiter die Frage, ob irreversible Fermentschädigungen für den bakteriziden Effekt verantwortlich sind. Wenn diese Möglichkeit auch nicht ganz auszuschliessen ist, so ist sie als alleinige Erklärung unzureichend. Hydrolytisch wirkende Fermente werden nicht so stark gehemmt wie dehydrierende Enzyme. Die

[1] N. CHUDIAKOW, Zbl. Bact., 2. Abtlg. *4*, 391 (1898). – C. FOA, Atti R. Acad. Lincei *15*, 185 (1906); Zbl. Bact. 1. Abtlg. Ref. *40*, 185 (1907). – BERGHAUS, Arch. Hygiene *62*, 172 (1907). – H. LÜCK, Schweiz. Z. Path. allg. Bakt. *16*, 728 (1953).

[2] H. LÜCK, Schweiz. Z. Path. allg. Bakt. *16*, 728 (1953).

[3] L. MASSART, Arch. int. Pharmacodyn. Thérap. *60*, 48, 56 (1938). – PH. J. LUTERAN, M. LANGERON und J. MERY, C. r. Acad. Sci. *228*, 338 (1949). – H. LÜCK, Schweiz. Z. Path. allg. Bakt. (im Druck).

Zelle müsste daher in neuem Nährsubstrat und unter Atmosphärendruck nach einiger Zeit über ausreichende Energie verfügen, um die geschädigten Zellbestandteile zu reproduzieren.

| Erhitzungstemperatur °C | Keimzahlen in $10^5$/cm³ | |
|---|---|---|
| | Kontrolle | 8 atü $O_2$ |
| 50 | 280 | 220 |
| 55 | 100 | 21 |
| 60 | 5 | 1 |

(Die Bakterienaufschwemmung wurde in ein Wasserbad der angegebenen Temperatur gesetzt und so lange darin belassen, bis sie diese Temperatur erreicht hatte.)

Der schädigende Einfluss des Sauerstoffs muss daher am Gen-Apparat des Organismus angreifen oder solche Vorgänge irreversibel blockieren, die unmittelbar mit der Zellteilung zusammenhängen oder die Enzymbildung katalysieren.

Nach Untersuchungen anderer Autoren ist bekannt[1], dass Sauerstoff als Elektronenakzeptor bei organischen Farbstoffen dienen kann, wobei letztere ionisiert werden. Der Sauerstoff lädt sich dadurch partiell oder total negativ auf. Überträgt man diese Vorstellung auf das Eiweissmolekül *E* der Zelle und fasst die Oxydation allgemein als eine Abgabe von Elektronen auf:

$$4\,E + O_2 \rightarrow 4\,E^+ + \cdots 2\,O^{2-},$$

so resultiert eine ähnliche Wirkung, wie sie auch ionisierende Strahlen (ultraviolettes Licht, Röntgenstrahlen) besitzen. Eine solche Ionisation oder Elektronenabgabe hat naturgemäss weitere Strukturänderungen des Eiweissmoleküls zur Folge. Komprimierter Sauerstoff scheint auf die lebende Zelle eine ähnliche, wenn auch viel schwächere Giftwirkung auszuüben wie Wasserstoffperoxyd:

$$2\,E + O_2^{2-} \rightarrow 2\,E^+ + 2\,O^{2-}.$$

Die doppelt negativ geladene $O_2$-Gruppe des Wasserstoffperoxyds steht in bezug auf ihre Oxydationsstufe zwischen dem ungeladenen $O_2$-Molekül und dem doppelt negativ geladenen O-Atom des Wassers.

An welchem Teil der Zelle der Sauerstoff angreift, ist bisher unbekannt. Ob er auch in der Lage ist, Mutationen auszulösen, bedarf noch eingehender Untersuchungen.

H. Lück

*Dairy Industry Control Board, Windhoek, P.O.B. 520, Südwestafrika, 20. August 1953.*

*Summary*

In this article the writer suggests an explanation for the germicide effect of compressed oxygen. The production of poisonous products of metabolism can be ignored, because the bacterial development is normal after removal of the pressure. Lack of nutritive material through inhibition of metabolism and permanent damage to the enzymes is also not a satisfactory explanation. The possibility is discussed that oxygen as an acceptor of electrones creates an ionisation of important parts of the cell and resulting in the death of the bacteria. The effect seems to be similar but weaker than that of $H_2O_2$.

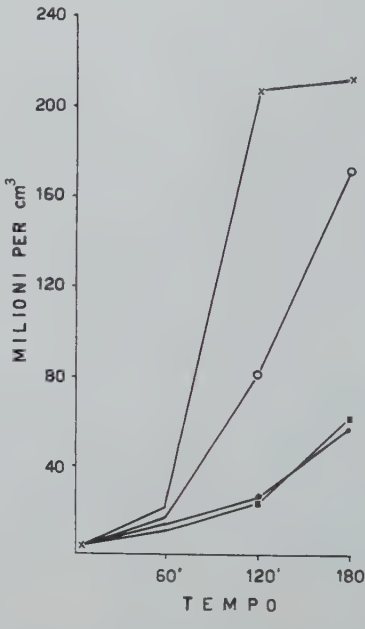
[1] G. Stein, Faraday Soc. Discuss. Radiation Chemist. *12*, 227 (1952). – J. Weiss, Trans. Faraday Soc. *42*, 133 (1946). – M. Evenari und G. Stein, Exper. *9*, 94 (1953).

## Inattivazione del profago a mezzo del calore

La esposizione istantanea e non graduale alla temperatura di 55°C, per 30–45 s, di cellule di ceppo lisogeno di *Micrococcus pyogenes aureus* determina la maturazione del profago a fago attivo (shock termico, Cavallo[1]) analogamente a quanto fanno i raggi ultravioletti nel noto fenomeno di Lwoff[2].

Se cellule bacteriche che hanno subito lo shock termico, vengono esposte alla temperatura di 45°C per 20 min, si ha un lieve ritardo nella velocità di maturazione, ma la quantità di bacteriofago, che viene prodotta, raggiunge quasi lo stesso valore che per cellule normalmente sottoposte a shock termico.

Se, invece, la esposizione a 45°C precede lo shock, non si determina più la maturazione del profago a fago. La esposizione a 45°C previene dunque l'effetto dello shock termico.




*M. pyogenes* 129. Prevenzione dell'induzione da shock termico a mezzo di esposizione a 45°C (numero di unità bacteriofagiche). • controllo, × shock termico, ■ esposizione a 45°C seguita da shock, o shock seguito da esposizione a 45°C.

L'esposizione a 45°C per 20 min non provoca alcuna alterazione a carico di bacteriofagi maturi, provenienti da culture lisogene di Micrococcus pyogenes.

Da questi risultati si può credere che la prevenzione a mezzo del calore dello effetto Lwoff si attui per inattivazione del probacteriofago.

Questi risultati possono essere ricollegati con le ricerche di Krueger sull'esistenza di un precursore intracellulare del bacteriofago nei germi sensibili al fago. Tale precursore è particolarmente sensibile al calore; esso è, infatti, completamente inattivato a 50°C in 2′30″, e a 45°C in 25′. Il comportamento delle costanti di inattivazione ha dimostrato che il costituente bacterico inattivato è una proteina simile al bacteriofago maturo (Krueger, Mecraken e Scribner[3]).

[1] G. Cavallo, C. r. Soc. Biol. *145*, 1885 (1951).

[2] A. Lwoff, L. Siminovitch e N. Kjeldgaard, Ann. Inst. Pasteur *79*, 815 (1950).

[3] A. P. Krueger, T. Mecraken e E. I. Scribner, Proc. Soc. Exp. Biol. Med. *40*, 573 (1939).

Krueger e Baldwin[1], nel 1937, sarebbero riusciti ad isolare da filtrati bacterici tale precursore, o almeno un materiale che lo contiene, per quanto tale estrazione non abbia sempre dato risultati regolari. Mentre in filtrati di germi lisati con penicillina o rotti a mezzo di ultrasuoni non era possibile trovare tale sostanza, recentemente invece Krueger è riuscito ad ottenere risultati significativi, servendosi di materiale ottenuto da germi in attiva riproduzione, lisati a mezzo di lisozima[2].

Le ricerche riferite sull'azione del calore, che saranno pubblicate altrove in extenso, sembrano indicare l'esistenza in *Micrococcus pyogenes* lisogeno di un componente intracellulare, corrispondente al precursore fagico di Krueger, e, come questo, sensibile al riscaldamento, e forse identificabile, o, almeno in stretta relazione, col probacteriofago.

G. Cavallo

*Istituto di Patologia generale dell'Università di Napoli, il 27 agosto 1953.*

*Zusammenfassung*

Die Erwärmung von lysogenen Staphylokokken auf 45°C während 20 min verhindert die Wirkung des thermischen Schocks sowohl auf die Bakteriophagen-Lysis wie auf diejenige bei niedriger Temperatur. Wird hingegen die Behandlung bei 45°C nach dem Schock durchgeführt, so bleibt sie wirkungslos.

Diese Ergebnisse lassen vermuten, dass in lysogenen Staphylokokken eine Komponente vorhanden ist, die den phagischen Präkursoren von Krueger enstpricht mit gleicher Wärmeempfindlichkeit. Identität mit dem Probakteriophagen ist möglich.

[1] A. P. Krueger e D. M. Baldwin, Proc. Soc. Exp. Biol. Med. *37*, 393 (1937).

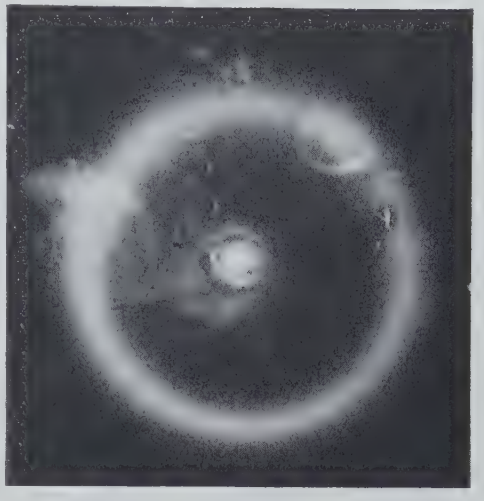
[2] A. P. Krueger, T. Cohn, P. N. Smith e C. D. McGuire, U. S. Naval Med. Bull. *48*, 510 (1948).

## On the Pigment of *Chaetomium* sp., the Cause of Green Stain of Heart-Wood in *Fagus sylvatica*

An ivy green coloration of the "false" heart-wood is not infrequently observed in the beech (*Fagus sylvatica* L.) from Carpathia. The wood is infected sometimes by *Ceratostomella fagi*[1], but the fungus responsible for the stain belongs to the genus *Chaetomium*. The latter species gave positive Bavendamm's reaction when tested on tannic acid medium for the presence of oxidases (Figure).

An alcoholic extract from a mass of perithecia of *Chaetomium* sp., gave a transient green colour which changed eventually to a brownish orange tint within a week. When preserved in an airtight dark glass, the solution did not change colour. The green-coloured extract showed a brightly green fluorescence under ultraviolet rays. The pigment is extractable also with ether and carbon disulphide, while chloroform and benzene do not dissolve it. The solution becomes colourless when treated with strongly oxidizing agents ($H_2O_2$). In order to obtain larger amounts of crude pigment, a mass of perithecia was extracted in a Soxhlet extractor with ethyl ether. The pigment obtained in this way is oily in consistence and corresponds in colour to the number 274 (yellow) of Séguy[1]. With $FeCl_2$ it gives a dark greenish brown colour.

A chemical investigation of the pigment (extracted with ether) indicated that the substance is related to



toluquinol[2]. Its properties much resemble those of the flavoglaucin which was isolated from the mycelium of the fungus *Aspergillus glaucus* Link ex Fr. by Gould and Raistrick[3]. The naturally oxidized pigment, brownish orange in colour, is comparable with another pigment, the auroglaucin, isolated likewise from *A. glaucus* by the same authors[3]. The colouring matters occurring in the body of *Chaetomium* sp. evidently play an important part in the life of the fungus,—like the original flavoglaucin and auroglaucin of *A. glaucus*–and constitute an oxidation-reduction mechanism in the organism. They may be considered as major end products of the carbohydrate metabolism[4]. The fungus *Chaetomium* sp. sometimes causes a red coloration of the wood, or, in culture, of the medium on which it is growing. This deep red discoloration might be due to two other pigments related to flavoglaucin and auroglaucin, namely erythroglaucin and rubroglaucin[5], the latter being only a mixture of erythroglaucin and physcion.

J. Paclt

*Biological Laboratory, Forest Products Research Institute, Bratislava, September 11, 1953.*

*Zusammenfassung*

Aus den Pilzfruchtkörpern von *Chaetomium* sp. ist ein Farbstoff isoliert worden, dessen Identität mit Flavoglauzin vermutet wird und welcher leicht in einen anderen, dem Auroglauzin nahestehenden Farbstoff übergeht.

[1] W. Loos, Arch. Mikrobiol. *3*, 370 (1932).

[1] E. Séguy, *Code universel des couleurs* (P. Lechevalier, Paris 1936).

[2] J. H. Cruickshank, H. Raistrick, and R. Robinson, J. chem. Soc. *1938*, 2056.

[3] B. S. Gould and H. Raistrick, Biochem. J. *28*, 1640 (1934).

[4] G. T. Johnson and B. S. Gould, Mycologia *45*, 172 (1953). – J. W. Foster, *Chemical Activities of Fungi* (Academic Press Inc., New York, 1949), p. 174.

[5] J. N. Ashley, H. Raistrick, and T. Richards, Biochem. J. *33*, 1291 (1939).

## Un nouveau type de chromosomes sexuels chez un mammifère

(*Ellobius lutescens* Thomas – *Rodentia – Microtinae*)

La sous-famille des *Microtinae*, dont 18 espèces sont cytologiquement caractérisées, offre un tableau cytologique cohérent: les nombres diploïdes varient de 30 à 62 et les nombres fondamentaux sont compris entre 50 et 64. Chez toutes les espèces, le ♂ possède un couple *X-Y* se disjoignant préréductionnellement. L'*X* est toujours grand, acrocentrique ou métacentrique; l'*Y* varie beaucoup: en forme de *V* à bras inégaux, ayant à peu près la taille de l'*X* dans certains cas (*Microtus oeconomus, Arvicola sherman*), il est très grand et acrocentrique chez *Microtus agrestis*, mais beaucoup plus souvent réduit à des dimensions très faibles, de 1 à 2 $\mu$.

Or, le Dr Balthazar (Institut Pasteur de Téhéran) auquel j'exprime ici ma reconnaissance, m'a envoyé plusieurs exemplaires d'un *Microtinae* fouisseur, remarquable par ses caractères adaptatifs très prononcés, yeux minuscules, oreille externe et queue rudimentaires, incisives proéminentes, *Ellobius lutescens* Thomas. La saison étant un peu avancée lorsque j'ai reçu cet intéressant matériel, la spermatogénèse touchait à sa fin et les observations qui suivent devront être complétées. Dès maintenant elles révèlent des faits extraordinaires.

Fig. 1.
Métaphase spermatogoniale.

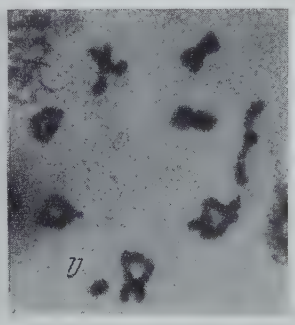



Fig. 2.
Métaphase auxocytaire.

La lettre *U* désigne l'élément impair univalent. Feulgen. × 1500.

J'ai examiné les testicules de 4 ♂♂ adultes. Le nombre 2 *N* est égal à 17 (Fig. 1) et l'assortiment chromosomique comprend: 1 paire de grands *V* à bras inégaux (1/2); 1 paire de grands à bras très inégaux (1/4); 1 paire de *V*, plutôt petits à bras égaux; 5 paires d'autosomes acrocentriques (1/5 à 1/6); enfin, un très petit *V* symétrique et impair. Si nous négligeons les bras courts des éléments acrocentriques, le *N.F.* peut être évalué à 20. La métaphase I (Fig. 2) montre 8 tétrades et un univalent dont la ségrégation est probablement préréductionnelle. Il s'ensuit que les spermatozoïdes compteront 8 ou 9 chromosomes. Il est dès lors tentant de considérer le petit *V* impair comme un *X*: notre *Ellobius* aurait la formule ♂: $2N = 16 + X$; ♀: $2N = 16 + 2X$.

Avant d'aller plus loin, formulons les deux remarques suivantes: les nombres 17 et 20 sont les plus bas que nous connaissions chez un Euthérien; il n'y a aucun exemple certain de digamétie *X-O* chez un mammifère, à l'exception peut-être de *Microtus montebelli* qui devrait être réétudié[1]. J'ai examiné les ovaires et la moëlle osseuse de deux femelles: les mitoses de la lignée myéloblastique montrent de nouveau 17 chromosomes, dont le petit élément impair, nettement reconnaissable. Une seule cinèse (sur 25) montre 2 petits *V*, mais le nombre total est encore de 17. Enfin, une division n'avait que 16 chromosomes, le *V* impair étant présent. Une métaphase I, trouvée dans un ovocyte mûr, présente 8 tétrades et le petit chromosome de caractère apparemment univalent.

A moins que la moëlle osseuse ne dérive d'une lignée cellulaire ayant éliminé un chromosome *X*, ce qui est bien invraisemblable, ces faits sont extraordinaires et semblent indiquer un mécanisme de détermination sexuelle original et aberrant. Avant de formuler des hypothèses, l'analyse cytologique devra être poussée aussi loin que possible.

R. Matthey

*Laboratoire de zoologie de l'Université de Lausanne, le 17 septembre 1953.*

*Summary*

*Ellobius lutescens*, a vole, has a diploid number of 17 in both sexes. This was determined in the testes (♂) and in the bone-marrow (♀). The number is the lowest ever seen in an eutherian mammal and falls wide of the formulae of the other *Microtinae*.

Unless the myeloblastic cell line eliminates one *X*-chromosome in the female, which seems very unlikely, we have to deal with an entirely new type of sex-determination.

[1] K. Oguma, Cytologica *5*, 796 (1937).

## Telophase Behavior of Extranuclear Chromatin and Its Bearing on Telophase Changes in Chromosomes[1]

Acentric chromosome fragments differ from centric fragments in both time of onset and extent of telophase change in neuroblasts of grasshopper embryos[2] and in pollen of *Tradescantia paludosa*[3]. Herein reported are further observations on the aberrant telophase behavior of acentric fragments in neuroblasts as well as observations on the telophase behavior of portions of unbroken chromosomes which, under certain conditions, protrude from the nuclear region. These observations will be discussed with respect to their bearing on the problem of telophase changes in chromosomes.

A brief review of the normal course of telophase changes in chromosomes, as recognized in aceto-carmine stained preparations of neuroblasts of *Chortophaga viridifasciata* (De Geer) embryos, will facilitate description of the abnormal telophase behavior of extranuclear chromatin. The beginning of telophase change (Fig. 1, upper group of chromosomes) is manifest in the disappearance of both the sharp outline and the uniform intense staining which characterize the chromosomes at anaphase. As the cell progresses through early telophase the chromatin becomes more diffuse. The centromere regions, however, remain condensed (all centromeres are terminal). Although the nuclear membrane has not yet appeared, the region of the cell occupied by the chromosomes and the polar spindle material—the nuclear region—is distinct from the cytosome at early telophase. By middle telophase each chromosome consists of a

[1] Work performed for Atomic Energy Commission under Contract No. W-7405-eng-26.

[2] J. G. Carlson, Proc. Natl. Acad. Sci. U. S. *24*, 500 (1938).

[3] The author is grateful to Dr. C. P. Swanson for this observation

fuzzy central core surrounded by diffuse chromatin which mingles with that of adjacent chromosomes. The centromere regions are distinct but slightly irregular in outline. The nuclear membrane forms at the polar boundary of the nuclear region. By late telophase the chromatin, even that in the centromere region, is quite diffuse; the formation of the nuclear membrane is complete at the apolar boundary of the nuclear region.

Fig. 1.—Photograph of a grasshopper neuroblast and its small daughter ganglion cell at early telophase. In the neuroblast (upper cell) it will be noted that the chromosomes have lost the sharp outline and uniform intense staining which characterized them at anaphase. The chromosome fragments in the cytoplasm still have the appearance of anaphase chromosomes. (The cell was given 64 $r$ of X-rays at the preceding interphase or very early prophase.)

A large number of telophase cells in grasshopper embryos given 64 $r$ of X-rays and stained with aceto-carmine have been studied to determine the appearance of the chromosome fragments. All telophase neuroblasts which contain fragments have been found to exhibit an asynchrony in telophase change in the centric and acentric fragments. In early telophase cells, the X-ray-induced acentric fragments lying apart from the chromosome group are in an anaphase condition, whereas the centric fragments and the unbroken chromosomes are in an early telophase condition (Fig. 1). Middle telophase cells contain fragments in the anaphase to early telophase state. In late telophase cells acentric fragments have the appearance of early to middle telophase chromatin. No cells have been seen in which the chromatin of the acentric fragments is in late telophase or interphase state. These observations clearly show that the initiation and progression of telophase change are not the same for acentric fragments as for centric fragments and unbroken chromosomes.

Several telophase cells have been observed in which portions of the chromosomes protrude from the nuclear region. Chromosomes of neuroblasts X-irradiated in late prophase tend to be slightly sticky at the subsequent anaphase (all other aspects of the cell are normal). Although this slight stickiness rarely results in breakage of the chromosomes[1], it sometimes causes several of the sister chromosomes to be pulled out so that their distal ends project beyond those of the other chromosomes. Figure 2 is a photograph of an early-middle telophase neuroblast, which had sticky chromosomes at anaphase, and its small daughter ganglion cell. The condensed appearance of the chromatin in the ganglion cell at telophase is characteristic of the differentiation which occurs in the formation of this cell. Possible factors contributing to the condensed state of the ganglion cell chromatin and its significance will be discussed in another report. In this report only the chromatin in the neuroblast will be considered.

Figure 2 shows that the projecting distal portion of each of the three pulled-out chromosomes in the neuroblast is only beginning to undergo telophase change whereas the proximal portion is in the early to middle telophase condition. Transition from the intensely staining, condensed type of chromatin in the distal portion to the less intensely staining, diffuse type in the proximal portion occurs at the edge of the nuclear region. The diffuseness of the chromatin within the nuclear region is associated with a swelling of the chromosomes—the proximal portion of each pulled-out chromosome begins to spread out inside the boundary of the nuclear region. At the centre of the nuclear region the swelling is of such degree that adjacent chromosomes merge, making the individuality of the chromosomes less distinct. The centromere regions have changed very little from the anaphase condition.

Thus we see that those portions of chromosomes, both centric and acentric, which lie outside the nuclear region do not undergo telophase change at the same time or to the same extent as do those portions within the nuclear region. This suggests that the factor or factors mainly responsible for changes in chromosomes at telophase are located within the nuclear region of the cell. The evidence obtained from neuroblasts with protruding chromosomes further indicates that the chromosomes swell considerably during telophase.

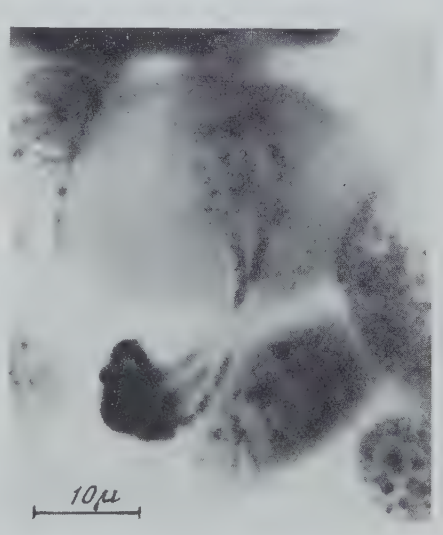



Fig. 2.—Photograph of grasshopper neuroblast and its small daughter ganglion cell in early-middle telophase. Note in the neuroblast cell (upper cell) the difference in extent of telophase change between the proximal and distal portions of the pulled-out chromosomes. (The cell received 64 $r$ of X-rays at the preceding late prophase.)

It is tempting to speculate that the intranuclear substance responsible, at least in part, for the swelling of chromosomes at telophase is the spindle material. Observations on living neuroblasts have revealed that the spindle in these cells arises from the karyolymph of

[1] Only about 0·01% of the chromosomes are broken when neuroblasts receive 64 $r$ of X-rays at late prophase.

the late prophase nucleus[1]. It may well be that during telophase spindle material becomes intimately associated with the chromatin, and that during prophase it is gradually released, as the chromosomes shorten, to give rise to "karolymph", which in turn gives rise to a spindle after nuclear membrane breakdown. That spindle material is in some way connected with telophase change can be further inferred from observations on cells in which the spindle has been altered experimentally. Hughes[2] and Gaulden[3] found that the behavior of the spindle material determines the time of onset of telophase change in cells recovering from complete destruction of a functional spindle by hypotony and by heat. If the spindle re-forms, onset of telophase change is delayed until the chromosomes have reached the poles of the spindle at anaphase. If, however, the spindle does not re-form, the chromosomes show telophase change immediately. It appears that telophase change occurs when the chromosomes are closely associated with disorganized spindle material, a situation which normally obtains at the beginning of telophase.

The role of the chromosome itself in the initiation and progression of telophase change remains obscure. The evidence herein presented shows, however, that the chromosomes are not solely responsible for the changes which are manifest in them at telophase, but that they are dependent to some extent on other factors.

Mary Esther Gaulden

*Biology Divison, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A., June 21, 1953.*

*Zusammenfassung*

Extranukleares Chromatin, in der Form azentrischer Fragmente oder Teile ungebrochener Chromosomen, welche aus der Kernregion von Heuschreken-Neuroblasten herausragen, unterliegt nicht gleichzeitig mit dem intranuklearen Chromatin den telophasischen Veränderungen. Der oder die für die Telophaseveränderungen vor allem verantwortlichen Faktoren scheinen in der Kernregion der Zelle lokalisiert zu sein. Es werden Belege dafür gegeben, dass die Chromosomen während der Telophase sich bedeutend vergrössern, und es wird die Ansicht vertreten, dass im Spindelmaterial, zum Teil wenigstens, die für die Schwellung der Chromosomen verantwortliche Substanz zu sehen ist.

[1] J. G. Carlson and A. Hollaender, J. Cellular Comp. Physiol. *31*, 149 (1948). – M. E. Gaulden and J. G. Carlson, Exptl. Cell Res. *2*, 416 (1951).
[2] A. Hughes, Quart, J. Microscop. Sci. *93*, 207 (1952).
[3] M. E. Gaulden, J. Tenn. Acad. Sci. *23*, 162 (1948).

---

## Analyse de l'induction neurale par autoradiographie

Les expériences de Brachet[1] ont montré clairement, quoique de manière indirecte, que l'induction neurale s'accompagne d'une migration de granules, colorables au rouge neutre, qui passent du chordoblaste dans le neuroblaste.

Il nous a semblé utile d'étendre ces expériences en utilisant la méthode autoradiographique pour la mise en évidence d'une éventuelle diffusion des macromolécules marquées de l'organisateur vers l'ectoderme.

[1] J. Brachet, Exper. *6*, 56 (1950).

Nous avons, dans ce but, utilisé la technique des émulsions nucléaires qui permet une localisation précise et une estimation quantitative de l'isotope tant dans le greffon que dans l'hôte.

Plusieurs séries d'expériences ont porté sur des embryons de Batraciens de différentes espèces (Pleurodèles, Axolotl, *Rana fusca*, *Rana temporaria*); elles ont consisté, soit en greffes d'organisateurs marqués dans des embryons entiers, soit en explantations: dans ce cas le neuroblaste marqué a été accolé à de l'épiblaste non marqué et vice versa.

Un essai préliminaire a montré que le glycocolle pénètre mal dans les embryons entiers conformément aux résultats déjà obtenus par Friedberg et Eakin[1] et par Brachet et Brygier[2] au moyen d'autres méthodes. Nous avons donc placé des organisateurs isolés dans le milieu radioactif.

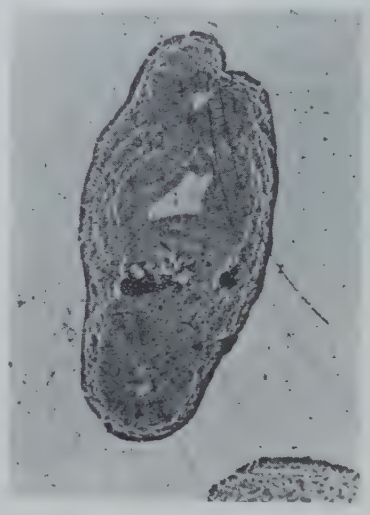


Fig. 1. Induction obtenue par la greffe d'un organisateur de *Rana fusca* dans un embryon d'Axolotl. – La préparation histologique est recouverte d'émulsion photographique puis colorée au vert de Méthyle-Pyronine.

Les images les plus convaincantes ont été obtenues dans les cas où l'hôte et le greffon provenaient d'espèces différentes et où ils étaient donc aisément reconnaissables. En voici un exemple: les organisateurs de jeunes gastrulas de *Rana fusca* ont été prélevés stérilement et soumis à un séjour de 3 h dans de la solution de Holtfreter stérile additionnée de 0,5 $\mu$M/cm$^3$ de glycine-I-$_{14}$C.

Après lavages soigneux et répétés dans du Holtfreter contenant du glycocolle non marqué, les greffons de *Rana fusca* ont été implantés dans des blastulas avancées d'Axolotl. Les embryons ont été fixés après 2 et 3 jours de développement, puis enrobés et coupés à 10 $\mu$ d'épaisseur.

Les coupes ont été alors recouvertes d'émulsion photographique $G_5$ Ilford «in gel form», suivant la technique exposée antérieurement[3]. Après 3 à 5 jours d'exposition en chambre noire, les préparations ont été développées, fixées et colorées à travers la gélatine par la méthode d'Unna (Fig. 1).

Dans les cas positifs (50%), on observe une radioactivité intense dans le greffon (Fig. 2). Une activité significative se manifeste aussi dans le système nerveux

[1] F. Friedberg et R. M. Eakin, J. exp. Zool. *110*, 33 (1949).
[2] J. Brachet et J. Brygier (communication personnelle).
[3] A. Ficq, F. Gavosto et M. Errera, Exper. Cell. Res. (sous presse).

induit et dans la chorde, quand celle-ci existe; cette activité est très nettement localisée dans les noyaux (Fig. 3). Une activité moindre s'observe dans le système nerveux primaire; en général, on retrouve des traces d'électrons dans toutes les régions de l'embryon où la morphogénèse est active. Dans les cas où le greffon s'est autodifférencié, il présente une activité remarquable, dans la chorde notamment.

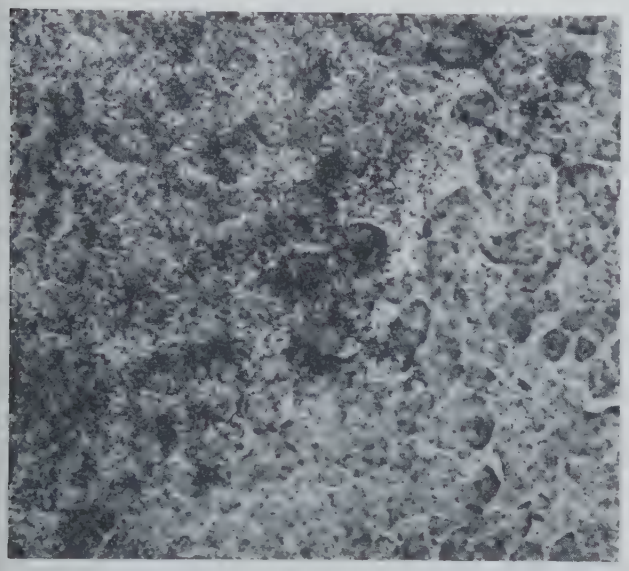


Fig. 2. Organisateur d'Axolotl marqué à la Glycine-I-$_{14}$C accolé à de l'ectoblaste. On observe une intense radioactivité dans les noyaux du greffon.

Pour distinguer la radioactivité de la glycine incorporée respectivement par les protéines et par les acides nucléiques, une série de coupes alternantes ont été hydrolysées par l'HCl N, pendant 5 min, à 60°, suivant la méthode de VENDRELY[1]. Une diminution de la radioactivité, qui atteint 50% de la valeur initiale, s'observe tant dans le greffon que dans les organes en formation.

Pour éliminer spécifiquement l'activité imputable à l'acide ribonucléique, nous avons traité une série de coupes à la ribonucléase selon la méthode de BRACHET[2]: l'autoradiographie de ces préparations accuse une diminution de 32% des traces. On peut en conclure que, lors de l'hydrolyse par l'HCl, 18% des traces éliminées proviennent du D.N.A. et d'une petite fraction protéique. Ce résultat se retrouve aisément en traitant des coupes à la ribonucléase d'abord, puis à l'HCl N à 60° pendant 5 min.

Une autre série d'expériences a été effectuée sur le même matériel et dans des conditions analogues, l'organisateur étant marqué, cette fois, par de l'acide orotique 2-$_{14}$C-6-carboxylique.

L'incorporation dans le greffon est sensiblement moindre que dans le cas de la glycine, mais la radioactivité induite se retrouve dans les mêmes régions à morphogénèse active; les noyaux sont particulièrement actifs et on y remarque assez souvent des traces émises par les nucléoles. Quelques électrons épars s'observent cependant entre les plaquettes vitellines.

Lorsque les coupes de cette série sont soumises à l'action de la ribonucléase, la radioactivité devient de l'ordre de celle du back-ground: l'incorporation, dans ce cas, se produit donc spécifiquement dans l'acide ribonucléique.

Nous avons tenté, enfin, quelques expériences d'induction dans le cas d'explantats en accolant des organisateurs d'Axolotl avec de l'ectoblaste de *Rana temporaria*, après avoir marqué à la glycine-I-$C_{14}$ soit l'un, soit l'autre des fragments.

On observe alors une forte radiactivité dans les noyaux du fragment traité et une diffusion modérée de la glycine vers l'ectoblaste ou le neuroblaste accolé. Toutefois, nous n'avons pas obtenu, dans ces conditions, de réponses morphologiques nettes, même dans le cas de témoins où l'explantat n'était pas radioactif.

Les coupes traités à la ribonucléase et à l'HCl N à 60° pendant 5 min, montrent des diminutions d'activité analogues à celles qui ont été observées dans les embryons entiers.

La glycine libre est, semble-t-il, totalement éliminée des greffons par les lavages au glycocolle non marqué: en effet, des fragments bouillis, traités de la même manière que les embryons en expérience, ne présentent qu'une radioactivité tout à fait négligeable, conformément d'ailleurs aux résultats de FRIEDBERG et EAKIN[1].

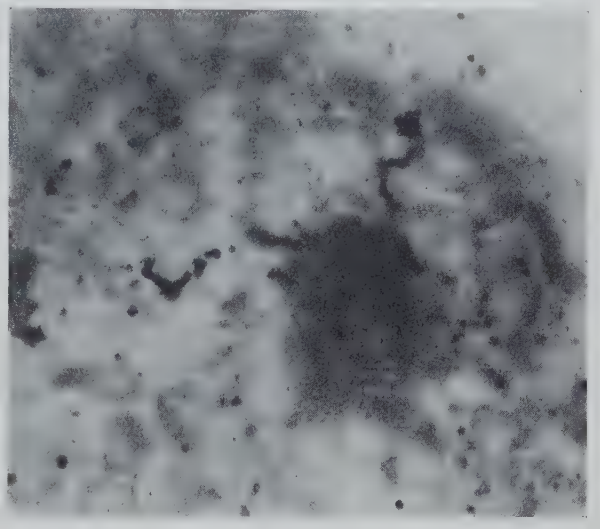


Fig. 3. Deux électrons émis par un noyau.

On peut donc attribuer la radioactivité observée dans les autoradiographies à des substances de poids moléculaire élevé, qu'il s'agisse d'acides nucléiques ou de protéines. Il faut dès lors conclure que l'organisateur, qu'il soit marqué par l'acide orotique ou par le glycocolle, laisse diffuser de telles substances dans les organes qu'il induit.

(Mme) A. FICQ

*Laboratoire de morphologie animale, Université de Bruxelles, le 7 juillet 1953.*

*Summary*

Neural induction is accompanied by a migration of granules from the organizer to the reacting ectoblast (BRACHET). The radioautographs of embryos or explants of amphibians seem to show that such a migration to the induced organs takes place when a marked organizer is grafted in a non-radioactive host.

Substances of high molecular weight (proteins or nucleic acids) diffuse from an organizer tagged either with radioactive glycine or with radioactive orotic acid.

[1] R. VENDRELY et F. LIPARDY, C. r. Acad. Sci. *223*, 342 (1946).
[2] J. BRACHET, Arch. Biol. *53*, 207 (1942).

[1] F. FRIEDBERG et R. M. EAKIN, J. exp. Zool. *110*, 33 (1949).

## Susceptibility to *Haemophilus influenzae* Infection in Relation to the Age Factor

Generalized infections with *Haemophilus influenzae* in man are most frequent during the first two years of life[1]. The higher resistance of later age groups is usually interpreted as the result of humoral immunity due to bacteriolysins of amboceptor-complement type[2], which were found to increase with age[3]. However, the increase in antibodies becomes apparent at a later age than the age of decrease in case incidence. Thus, we have felt some doubt whether the humoral factor is solely responsible for the age-induced resistance, particularly as recent research on this problem in virus infections has shown that the interdependence of cause and effect is quite complicated[4]. As the mouse is not a natural host to true *H. influenzae*, at least acquired humoral immunity in the adult animals cannot be of importance. We, therefore, undertook to examine this problem by the inoculation of mice of different ages. The capsulated smooth *Haemophilus influenzae* strain type "B" used in our experiments had been isolated from the pericardial exudate of an infant who died from purulent pericarditis.

In 20 adult mice (average weight 25 g) the subcutaneous injection of half a slope blood agar culture caused only a local swelling which subsided after 1–2 days and all the animals remained alive. There was no damage to the skin and the fur at the site of injection. However, a transient bacteraemia quite often followed the injection. When groups of 6 mice were inoculated in the way described and killed after 3, 24 and 48 h, the injected organisms could be cultured after 3 h from the liver of 4 animals but in no instance after 24 and 48 h. Thus, the adult mouse quickly overcame this bacterial flooding and showed no subsequent damage.

*Table I*
Effect of *H. influenzae* infection in infant mice of different age

| Age in days | Number of mice | Dead within 48 h | Recovered |
|---|---|---|---|
| 5 | 20 | 10 | 10 |
| 10 | 20 | 13 | 7 |
| 15 | 20 | 3 | 17 |
| 20 | 20 | 2 | 18 |
| 30 | 20 | 0 | 20 |

In infant mice, the infection took quite a different course. Groups of 20 mice were subcutanously injected with 1/50 slope agar culture per gram body weight at the age of 5, 10, 15, 20, and 30 days. The results are given in Table 1. About half of the animals in the first two age groups died quickly and the bacteria could be recovered from liver and spleen of all the dead animals. The histological examination of the internal organs revealed necrotic foci in the liver with marked leucocytic infiltration. In the two later age groups the death rate dropped considerably and became nil in the last one.

The survivors of the first group, still naked and blind at the time of infection, were markedly stunted in their general development and showed inhibition of the growth of hair over a wide area around the site of injection as well as similar smaller defects on other parts of the fur (Fig.). Histological examination of the affected skin, ten days after injection, revealed a heavy polymorphonuclear infiltration of the subcutis, thickening of the derma and, in places, rarefaction of the epidermis with blocking of the hair follicles by keratin plugs (follicular hyperkeratosis). Three weeks later, the infiltration had diminished and young hairs were growing, but the cutis was still thickened. This skin involvement, analogous to the dermo-necrotic effect of *H. pertussis* toxin[1], was obviously due to endotoxins, liberated from the bacteria, against which the adult animal appears to be more resistant. The skin lesions cleared after about one month but a certain irregularity of the hairy coat remained visible. In the other age groups, where the fur had already started to grow at the time of infection, no such effect on the skin was observed but the general development in the second age group was also markedly retarded.

Infant mouse, 10 days after infection, injected with *H. influenzae* in right hindquarters at the age of 5 days. Large bald area over posterior part of body, a smaller one above the right eye. General retardation of growth, as compared with control mouse of same age and stock behind it (Natural size).

These experiments demonstrate that infant mice are considerably more sensitive to Haemophilus infection than older ones. After the 15th day of life the resistance approaches that of the adult and reaches that level at the 30th day.

The pathogenicity to embryos is still higher than to postnatal animals. As far back as 1937, Gallavan[2] reported irregular pathogenicity of *H. influenzae* to chick embryos by chorio-allantoic inoculation, which caused encephalitis, meningitis and occasional ectodermal necrosis. Gallavan and Goodpasture[3], inoculating *H. pertussis*, observed also ectodermal necrosis, increase of mesodermal cells and pulmonary lesions reproducing those of human pertussis. Shaffer and Shaffer[4] demonstrated the marked superiority of yolk sac inoculation over the chorio-allantoic route with *H. pertussis*.

Our Haemophilus strain killed chick embryos by yolk sac inoculation regularly in less than 24 h, and corresponding effects were observed in mice. 8 females in the middle of pregnancy were injected subcutaneously with half a slope agar culture and all aborted within 36 h but remained otherwise healthy. Out of 8 others, in-

[1] T. M. Rivers, Amer. J. Dis. Child. *24*, 102 (1922).
[2] J. Wright and H. K. Ward, J. Exp. Med. *55*, 235 (1932).
[3] L. D. Fothergill and J. Wright, J. Immunolog. *24*, 273 (1933).
[4] M. M. Sigel, Ann. Rev. Microbiol. *6*, 247 (1952).

[1] M. R. Pinto, Arquivos Inst. Câmara Pestana *1*, 55 (1943). – H. Violle, Bull. Acad. nat. Méd. *134*, 518 (1950).
[2] M. Gallavan, Amer. J. Pathol. *13*, 911 (1937).
[3] M. Gallavan and E. W. Goodpasture, Amer. J. Pathol. *13*, 927 (1937).
[4] M. F. Shaffer and L. S. Shaffer, Proc. Soc. Exp. Biol. Med. *62*, 244 (1946).

jected towards the end of pregnancy, two aborted and six delivered sickly litters. Many of these baby mice were eaten by their mothers but a few survived and showed patchy defects of the fur similar to those brought about by direct injection. The most regular damage of the aborted foetus, as revealed by histological examination, were scattered necrotic foci in the liver without leucocytic infiltration.

In a further experiment, 10 females in the middle of pregnancy were subcutaneously injected with half slope agar cultures and killed 24 h later. In six of them abortion had already begun by that time. Cultures were performed from the liver and spleen of the females on the one hand, and from the embryos on the other. No *Haemophilus* was found in the organs of the mother animals nor was there any subplacentar hemorrhage. The abortion could not, therefore, be related to maternal injury. Out of 66 embryos, 13 yielded a rich confluent growth, 8 showed isolated colonies and 45 were sterile. The distribution of these findings is given in table No. II.

*Table II*

Presence of *Haemophilus* in embryos of pregnant mice infected 24 h before examination

| Serial No. of female | Embryos | | | |
|---|---|---|---|---|
| | Total number | Confluent growth | Isolated colonies | Sterile |
| 1 | 7 | 7 | — | — |
| 2* | 6 | — | 1 | 5 |
| 3* | 4 | — | 4 | — |
| 4 | 10 | — | — | 10 |
| 5* | 7 | — | — | 7 |
| 6* | 8 | 6 | 2 | — |
| 7* | 6 | — | — | 6 |
| 8 | 7 | — | — | 7 |
| 9 | 4 | — | — | 4 |
| 10* | 7 | — | 1 | 6 |
| Total | 66 | 13 | 8 | 45 |

* Abortion had already started before examination.

The bacteriological findings of the placentas, cultured separately, did not differ from those of the corresponding foetus. Experiments with intravenous injections of 1/10 slope agar cultures gave even more impressive results. After 24 h, the organs of all the females were sterile whilst the Haemophilus could be recovered from the embryos in most of the litters.

In conjunction with our previous results, these findings may be interpreted as follows: The embryos are killed by the liberated endotoxins to which they are more sensitive than the adult animals. If bacteria penetrate through the placental barrier into the foetus, they establish a foothold in the impaired embryonic and placentar tissue, whilst they are quickly destroyed in the adult animal by cellular defence mechanisms. Humoral antibodies would have acted equally in both, mother and foetus, since they pass unhindered through the placenta in rodents, apes and man[1].

The susceptibility of infants to *H. influenzae* infections cannot, therefore, be explained solely by a lack of humoral immunity. The primary cause appears to be a particularly high vulnerability of the immature organism to the endotoxins of Haemophilus which seem to paralyze its cellular defence. The exact nature of this process still requires elucidation. It would be even more interesting to investigate, whether a difference in vulnerability, similar to that between foetus and mother, pertains also to other immature tissues within a mature organism, i.e. in tumor-bearing animals. Should this be confirmed, the Haemophilus toxin might be considered for trial as an anticancerous agent.

I wish to express my thanks to Dr. A. Komaroff, Director, Govt. Veterinary Virus Laboratory Haifa, for his kind help in the experiments with chick embryos and to Dr. B. Geley, chief, Pathol. Section, Govt.Hospital "Rambam", for the histological examinations.

R. Reitler

*Government Hospital «Rambam» (Maimonides), Haifa, Israel, July 17, 1953.*

*Zusammenfassung*

Mäuse zeigen in den ersten zwei Wochen ihres Lebens, im Vergleich mit erwachsenen Tieren, eine ähnlich erhöhte Empfindlichkeit gegenüber Allgemeininfektion mit *Haemophilus influenzae* wie menschliche Kleinkinder in den ersten zwei Jahren. Diese Erscheinung beruht vermutlich in erster Linie auf einer besonders hohen Empfindlichkeit des infantilen Organismus gegenüber den Endotoxinen, welche die zellulären Abwehrfunktionen hemmen. Noch empfindlicher sind Föten in der Mitte der Gestationsperiode, die sämtlich abgetötet werden, während das Muttertier keinerlei Schädigung erleidet.

Es wird angeregt, zu untersuchen, ob eine ähnliche Verschiedenheit der Empfindlichkeit zwischen den unreifen Zellen von malignen Tumoren und den reifen ihrer Träger besteht.

## Der Rucktanz als wesentlicher Bestandteil der Bienentänze

Vor einigen Jahren berichteten wir über richtungsweisende Bienentänze bei Futterplätzen in Stocknähe[1]. Es stellte sich heraus, dass bei einer Entfernung des Futterplatzes von zum Beispiel 30 m sowohl Sicheltänze (Baltzer, Tschumi) als Acht- und Schwänzeltänze (v. Frisch) nebst Übergangstanzformen ausgeführt wurden. Dazu kamen noch regellos eingestreute Rundtänze und ungerichtete Sicheln. Trotz der starken Variation schienen die Tänze, wie dies auch Tschumi[2] feststellte, eine ausgesprochen richtungsweisende Funktion zu erfüllen. Letztere kann deshalb unseres Erachtens nur auf den im Jahre 1950 von uns beschriebenen «Rucktänzen» beruhen, die unter anderem auch die «Wendepunkte» Baltzers[3] markieren. *Nur diese Rucktanzstrecken können durch Länge und Richtung die Lage der Futterquelle anzeigen,* entsprechend den von v. Frisch entdeckten Regeln. Die zwischen den Rucktänzen eingeschalteten Laufstrecken haben, ebenso wie die Rundtänze und die ungerichteten Sicheln, eine aktivierende Bedeutung. Ihr Auftreten ist einerseits durch örtliche Umstände auf der Wabe bedingt, andererseits durch das Temperament des Volkes. Letzteres kann verschieden sein, und zwar sowohl *vorübergehend* durch Einflüsse, wie Wetter, Qualität der Futterquelle, Stärke und Entwicklungszustand

[1] J. H. Mason, T. Dalling, and W. S. Gordon, J. Path. Bact. *33*, 783 (1930).

[1] G. Hein, Exper. *6*, 142 (1950).

[2] P. Tschumi, Rev. suisse Zool. *57*, 584 (1950).

[3] F. Baltzer, 12. Jber. Schweiz. Ges. Vererbungsforsch. *27*, 197 (1952).

des Volkes, Alter der Königin, als auch *bleibend* verschieden durch erbliche Unterschiede zwischen den Rassen oder einzelnen Völkern.

G. Hein

*Institut für vergleichende Physiologie der Universität Utrecht, den 11. August 1953.*

*Summary*

Bee dances indicating the position of feeding places situated at short distances from the hive show a great variability. They have only one element in common which seems suitable to this purpose: the "pull-dance" (Rucktanz), described by us earlier.

## Method for the Quantitative Evaluation of Platelet Function in Blood Coagulation

The role of platelets in blood coagulation has been characterized in connection with the identification of several active agents supplied by the platelets themselves: (1) platelet thromboplastic factor[1]; (2) platelet factor 1, presenting Ac-globulin activity[2]; (3) platelet factor 2, which accelerates the conversion of fibrinogen into thrombin[2]; (4) platelet factor 3, or antiheparinic factor, which is probably similar or identical with the thromboplastic factor[3].

Quantitative or qualitative alterations of platelets may be revealed by the incomplete transformation of prothrombin into thrombin (positivity of the prothrombin consumption test). This test is, however, positive also in various other conditions, and its limited specificity is often the cause of diagnostic difficulties.

For the quantitative evaluation of platelet function as a whole, a method has been worked out which is based on the functional study of isolated platelets in a clotting system. The principle of the method is the following: all factors are kept constant, except the factor to be determined, i.e. platelet functional activity, according to this scheme:

*Constant*
Prothrombin
Antihemophilic globulin and allied factors
Ac-globulin or proaccelerin
Factor VII or proconvertin or SPCA
Calcium
Platelet number (300,000/cu.mm)

*Variable*
*Platelet functional activity*

The substrate containing all factors to be kept constant is obtained by preparing a strictly platelet-free plasma (high speed centrifugation, cold, siliconed glassware and needles[4]). Calcium is supplied in known amounts.

The most important point of the method is the preparation of the platelet suspension. In previous research, exact quantitative tests have not been employed[1], partly because the preparation of non-agglutinated platelet suspensions containing a fixed amount of platelets presents some technical difficulties. To avoid agglutination and to perform an accurate count of the platelets, it is indispensable to use throughout: (*a*) arquad coated needles; (*b*) siliconed coated glassware; (*c*) sequestrene as anticoagulant; (*d*) triton and sodium acetate for preserving the platelets. The details of this technique are described by Stefanini *et al.*[2] and have been followed exactly. Differential centrifugation and repeated washings of platelets have to be carried out rapidly.

By employing such methods, it is possible to prepare suspensions of non-agglutinated platelets and to bring their concentration to 300,000/cu.mm with sufficient accuracy. The test for the quantitative evaluation of platelet function in blood coagulation is carried out as follows; 0·1 $cm^3$ of the platelet suspension is added to 0·1 $cm^3$ of platelet-free plasma. One tenth $cm^3$ of $CaCl_2$ 0·025 *M* is added to this mixture and the clotting time at 37°C is recorded. By using serial dilutions of the platelet suspension, a standard curve is obtained which makes it possible to evaluate the percentages of platelet activity. The recalcification time of the platelet-free plasma, to which the undiluted platelet suspension has been added, is considered as 100%. If saline is added instead of platelet suspension, the values of the recalcification time refer to 0% platelet activity, and should be more than 6–8 min at least. The percentage of reduction of the clotting time is converted into percentage of platelet functional activity (Fig. 1).

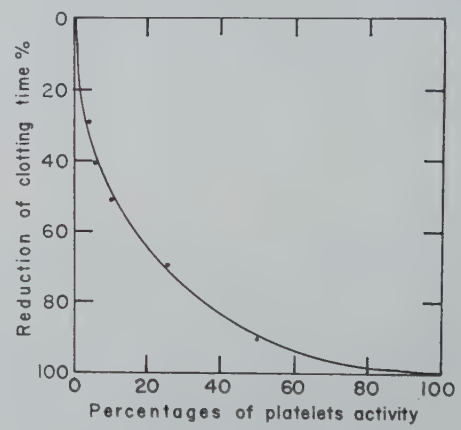



Fig. 1.–Standard curve for the evaluation of the percentages of platelets activity from the reduction of clotting time.

The same test might be carried out by using a two-stage method, as already observed by one of us[3]. High concentrations of purified prothrombin (3000 units/$cm^3$), platelet-free plasma and optimal calcium amounts are used for this purpose. Platelet suspensions may be prepared according to the same procedure.

Preliminary observations carried out by using this method[4] have shown that variations of platelet func-

[1] M. Stefanini, Amer. J. Med. *14*, 64 (1953).
[2] A. G. Ware, J. L. Fahey, and W. H. Seegers, Amer. J. Physiol. *154*, 140 (1948).
[3] S. Van Creveld and M. M. P. Paulssen, Lancet *2*, 242 (1951).
[4] J. Bernard, J. L. Beaumont, and M. Cl. Charreyron, Rev. Hématol. *8*, 20 (1953). – J. P. Soulier and M. J. Larrieu, J. Lab. Clin. Med. *41*, 849 (1953).

[1] J. Bernard, J. L. Beaumont, and M. Cl. Charreyron, Rev. Hématol. *8*, 20 (1953). – J. P. Soulier and M. J. Larrieu, J. Lab. Clin. Med. *41*, 849 (1953). – A. J. Quick, W. F. Stapp, and C. V. Hussey, J. Lab. Clin. Med. *39*, 142 (1952). – L. N. Sussman, N. Wald, and R. L. Rosenthal, Blood *7*, 1100 (1952).
[2] M. Stefanini, W. Dameshek, J. B. Chatterjea, E. Adelson, and I. B. Mednicoff, Blood *8*, 26 (1953). – M. Stefanini and W. Dameshek, New England J. Med. *248*, 797 (1953).
[3] S. A. Johnson and P. de Nicola, Unpublished observations.
[4] P. de Nicola, P. Rosti, and C. Carcupino, Proceed. IV. Congr. European Soc. Hematol., Amsterdam, Sept. 8–12, 1953.

tional activity as a whole can be detected not only in thrombocytoasthenias but also in other conditions, as for instance during menses[1]. (See previous researches on the positivity of the prothrombin consumption test during menses[2].) In such cases, as well as in other conditions, significant modifications can be detected, regardless of the actual platelet count, which might influence the results in other methods.

P. de Nicola and P. Rosti

*Department of Internal Medicine, University of Pavia, October 16, 1953.*

*Zusammenfassung*

Eine Methode zur Auswertung der funktionellen Plättchenaktivität wird beschrieben. Isolierte Plättchen in konstanter Zahl (300000/cu.mm) werden einem alle übrigen Gerinnungsfaktoren in konstanter Menge enthaltenden Substrat zugesetzt. Prozente können von den Gerinnungszeiten durch eine Eichkurve ermittelt werden.

[1] P. de Nicola, P. Rosti, and C. Carcupino (in press).

[2] A. Baserga, P. Rosti, and R. Furian, Lancet *2*, 460 (1950). – P. Rosti, and R. Furian, Haematologica *35*, 237 (1951). – P. de Nicola, Canad. M. A. J. *67*, 623 (1952).

---

## The Influence of the Nucleus and of Heat Regulation on the Dipeptidase Activity of the Nucleated and Anucleated Erythrocytes of Vertebrates

*Researches in Comparative Cytochemistry*

The study of the peptidases—hydrolytic enzymes which split the CO-NH bond of the amino acids combined to form the peptides—has been greatly advanced by the work of the Carlsberg Group (Linderström-Lang, Holter an others[1]). These workers have put forward the hypothesis that these enzymes, known to be ubiquitous, may play a part in the process of protein synthesis which occurs in the cells; such a suggestion has arisen from the fact that the peptidases are found in very considerable quantities in cells with notable power of synthesis (Duspiva[1], Smith[2]). It has moreover been possible to locate the peptidases in the cytoplasm of the cells (Duspiva, Holter and Lövtrup[3]), and not in the nucleus. Urbani[4] was able to notice that in amoebas experimentally enucleated, the dipeptidases are partially affected by the removal of the nucleus, from which one must conclude that the nucleus, although it does not itself contain the enzyme, partly controls its presence in the cytoplasm.

We may recall briefly that researches on dipeptidase activity have even been carried out on the cells of human haematopoietic tissues, in normal conditions and in connection with the relation between *d-peptides* and malignant processes (Merten, Adams, Salvidio, and Parreira[1]). Recently Adams *et al.*[2] have assayed Mammalian and Avian red blood corpuscles for four separate peptidases; they found that each peptidase varied only a 3-fold range from species to species, and that nucleated avian erythrocytes showed no distinctive differences in activity from mammalian corpuscles.

To explain more clearly the relationship between nucleus and cytoplasm, G. Cotronei suggested to us that a piece of comparative research, extended to all the Vertebrates, might be of interest in the evaluation of the influence of the nucleus on the enzymatic content of haematic cells.

We have therefore undertaken an analysis of the dipeptidases in the erythrocytes of the Vertebrates, from Fish to Man; we have, however, not been able for the moment to carry out research on the Cyclostomata, owing to lack of material.

*Material and Method.* Erythrocytes of the following species: *Carassius auratus, Triton cristatus, Bufo vulgaris, Rana esculenta, Lacerta viridis, Anser anser, Gallus domesticus, Mus musculus,* and *Homo sapiens.* Determination of the dipeptidase activity (substrate *dl*-alanylglycine 0·2 m) was carried out according to the titrimetric micromethod of Linderström-Lang and Holter[3]. After taking the sample the red corpuscles were washed, counted and hemolysed with distilled water. We calculated the standard cleavage of alanylglycine 100,000 cells and for one hour's incubation at 38°C, and the cleavage per cellular volume unit, obtaining this latter by means of a microhematocrit.

*Results.* Having ascertained that in the erythrocytes of the Urodela also, the enzymatic activity occurs almost entirely in the cytoplasm, we established the pH opimum of the peptidases in the various classes of Vertebrates, and by comparing them, determined the enzyme content of the erythrocytes of the Vertebrates examined.

As one can see from the table, the optimum pH in the various species examined is around 7·70, though it has an optimum of 7·40 in Fish, and 7·90 (with a secondary maximum at 7·10) in Man. Quantitatively, on a superficial examination of the data, it would seem that there exists a *direct proportionality* between cellular volume and dipeptidase activity. On a more careful examination, however, one notes that while the relationship between the volume of the erythrocytes of Triton and Mouse is 50 to 1 (2500 micron$^3$ to 51 micron$^3$), the relationship between the respective standard cleavages is 103 to 1, and the cleavage per volume is 1·46 for the Triton, 0·60 for the Mouse, and 0·41 for Man. These facts show that the enzymatic activity of the erythrocytes in Mouse and Man is *less* that one would have expected from the relationship to cellular volume. An examination of the connection between cellular volume and enzymatic activity per volume unit of the erythrocytes of the cold blooded animals shows, on the other hand, that the dipeptidase activity is proportional to the cellular size and practically equal in the various cold blooded species, in relation to the unit of cellular volume (seeTable). From the Figure it can be seen that the cleavage per volume unit (in grey) is almost the same in the cold blooded Vertebrates, while there is a clear difference between cold and hot blooded species. Between

[1] K. Linderström-Lang and H. Holter, Z. Physiol. Chem. *226*, 173 (1934).

[2] F. Duspiva, Biol. Zbl. *62*, 403 (1942). – E. L. Smith, Proteolytic Enzymes, in *The Enzymes*, ed. By J. B. Summer and K. Myrbäck, Vol. 1, second part (Academic Press Inc., New York, 1952).

[3] F. Duspiva, Biol. Zbl. *62*, 403 (1942). – H. Holter and S. Lövtrup, C. r. Lab. Carlsberg *27*, 2 (1949).

[4] E. Urbani, Arch. Int. Physlol. *60*, 189 (1952).

[1] R. Merten and M. Windschuh, Z. Vit. Horm. Ferm. Forsch. *1*, 35 (1947). – E. Adams, M. McFadden, and E. L. Smith, J. Biol. Chem. *198*, 664 (1952). – E. Salvidio and F. Parreira to be published in: Anais do Instituto de Medicina Tropical 1953, Oct.

[2] E. Adams, M. McFadden, and E. L. Smith, J. Biol. Chem. *198*, 664 (1952).

[3] K. Linderström-Lang and H. Holter, *Methoden der Fermentforschung*, Baumann and Myrbäck, (Leipzig 1941).

| No. of samples | Species | pH optimum | Cellular volume | Standard/Cleavage | Volume/Cleavage |
|---|---|---|---|---|---|
| 6 | Triton . . . . . | 7·70 | 2,500 | 270 | 1·46 |
| 1 | Rana . . . . . | 7·70 | 930 | 93 | 1·45 |
| 5 | Bufo . . . . . | 7·70 | 815 | 70 | 1·33 |
| 1 | Lacerta . . . . . | 7·70 | 290 | 33 | 1·40 |
| 3 | Carassius. . . . . | 7·40 | 225 | 27 | 1·50 |
| 1 | Anser . . . . . | 7·70 | 130 | 8 | 0·80 |
| 2 | Gallus . . . . . | 7·70 | 100 | 6 | 0·75 |
| 1 | Mus . . . . . | 7·70 | 51 | 2,6 | 0·60 |
| 3 | Homo . . . . . | 7·90 | 80 | 2,50 | 0·41 |

The cellular volume is in cubic micron; the standard cleavage and the cleavage per cellular volume unit (Volume/Cleavage) are in microliter HCl 0·06 N.

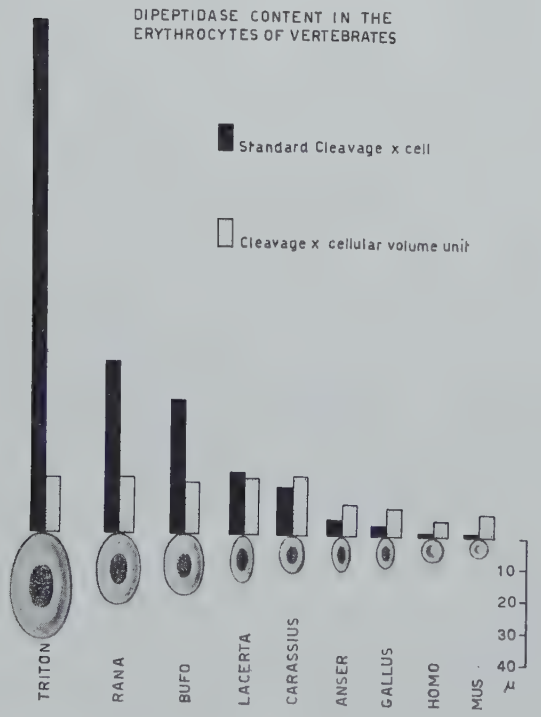
Birds and Mammals one sees that the cleavage/volume is greater for the former, and for Mammals it is the lowest found in the zoological scale. From an examination of these data we can establish the following points:

(1) The dipeptidases are present in the cytoplasm of erythrocytes, and absent from the nucleus.

(2) The quantity of peptidases in an erythrocyte is proportional to the volume of the erythrocyte itself.

(3) The quantity of dipeptidases per volume unit of cytoplasm is constant in cold blooded Vertebrates. In warm blooded animals, the quantity of enzyme per volume unit of protoplasm is less than in the cold blooded animals. It is also possible to differentiate the hot and cold blooded Vertebrates from the point of view of enzyme content, by comparing the enzymatic activity.

(4) In hot blooded Vertebrates with nucleated erythrocytes (Avians), and anucleated erythrocytes (Mammals) the enzyme content is less in Mammals, whose red blood corpuscles have lost the nucleus; moreover, the enzymatic content of the erythrocytes of Mammals is less than that of all other Vertebrates.




For a full discussion of the matter, reference should be made to another work in process of publication (Salvidio and Urbani[1]).

*Conclusions.* The comparative criterion on which this piece of research is based has permitted us to obtain objective results on the dipeptidase content of the erythrocytes in the range of Vertebrates, from Fish to Man, and it is the first time that this particular field of research has been studied. We have been able to distinguish the influence of the nucleus on the enzymatic content of the red blood corpuscles, and we have also been able to establish that there is a correlation between the enzymatic activity and the phenomena of heat regulation. Examination of the erythrocytes of five classes of Vertebrates has shown that from the enzymological point of view also, one can distinguish between Vertebrates with nucleated erythrocytes and Vertebrates with anucleated erythrocytes. Finally Avians, in which the erythrocytes present the morphological appearance (nucleus) of cold-blooded Vertebrates in a warm-blooded organism, have an enzymatic content quantitatively intermediate between that of Fish, Amphibians, and Reptiles from one side, and Mammals on the other.

E. Salvidio and E. Urbani

*Institute of Comparative Anatomy, University of Rome, August 18, 1953.*

*Riassunto*

Applicando il metodo comparativo gli autori hanno studiato l'influenza del nucleo sul tenore in dipeptidasi (substrato *d–l* alanilglicina) degli eritrociti di Vertebrati eterotermi ed omeotermi dai Pesci all'uomo. La mancanza di materiale non ha permesso di comprendere per il momento, nelle osservazioni gli eritrociti dei Ciclostomi.

Con il micrometodo di Linderström-Lang e Holter si è potuto localizzare l'enzima prevalentemente nel citoplasma degli eritrociti, inoltre si è potuto accertare:

I. La quantità di dipeptidasi di un eritrocita è proporzionale al volume dell'eritrocita stesso.

II. La quantità di dipeptidasi per unità di volume protoplasmatico è una costante negli eterotermi.

III. Negli omeotermi la quantità di enzima, per unità di volume protoplasmatico, è minore che negli

[1] E. Salvidio and E. Urbani, to be published in: Haematologica *37* (1953).

eterotermi. Il tenore enzimatico degli eritrociti dei Mammiferi è inferiore (a parità di volume protoplasmatico) a quello di tutti gli altri Vertebrati studiati.

Dall'insieme delle ricerche è risultata l'influenza del nucleo e della termoregolazione (dedotta dalle condizione eteroterme e omeoterme degli organismi studiati) sul tenore in dipeptidasi degli eritrociti nucleati e anucleati dei Vertebrati.

## Some Remarks on Two-Dimensional Paper Electrophoresis[1]

Paper electrophoresis has been making rapid development during recent times both on account of the simplicity of carrying out the method and the low cost of the equipment required. But to be sure that it is really paper electrophoresis and not just chromatography with applied voltage, the process should be carried out under conditions which ensure having a movement of the substance which depends essentially on the electric field applied, and exclude other factors which might interfer with the final result. From this point of view, flat electrophoresis presents undoubted advantages over that carried out on an inclined surface, even with continuous evaporation of the solvent according to MACHEBOEUF and his associates[2] (electrorheophoresis).

In any case, paper electrophoresis effectively opens up a large field of inquiry on account of the various opportunities for successful combination with chromatography[3], also for effecting a continuous separation[4] of the different substances and for carrying out two-dimensional electrophoresis[5], that is, twice successively in directions at right angles to each other and under different electrical conditions.

Our working conditions[6] for the study of two-dimensional paper electrophoresis have been the same as those described by KUNKEL and TISELIUS[7], with the exception of one particular: the two opposite sides of the square sheet of paper did not dip into buffer, but were electrically connected there with small siphons of the same paper (four at each side) not larger than 15 mm. This arrangement, even though it forced us to work with a lower current intensity, operated in such a way that the movement of the substances was independent of their position on the paper and due exclusively to the forces of the electric field. In fact, also in electrophoresis in a single direction, we have noticed many times that when the paper strip was dipping at the two extremities for its entire width into buffer contained in the vessels, there was—even without the current—a movement of the substances due to the strong influx of electrolyte. This movement was negligible in the central part of the strip and increased little by little gradually as it approached the buffer in the vessels. These movements had a really noticeable influence on the results of the electrophoresis, particularly for amino acids and the more diffusible ions. When, in order to have a larger path available, a starting point was selected near one of the ends of the strip, it happened sometimes that, at first, the substances migrated towards the nearest vessel (pole with the same charge) before starting their normal route towards the opposite pole; other conditions being equal, the path taken by the substances differed according to the initial point. Even when the starting point was chosen in the central part of the strip, during the electrophoresis with the movement due to the electric field, the substances fell under the influence of the attraction exerted by the buffer. Although it was possible to determine this influence exactly at each point, both without the application of the current and when the strip was inserted in the circuit (and in this case such influence adds up algebraically to the value of the electroendosmotic flow), we preferred to try to eliminate this interference. The use of narrow siphons (one each seven centimetres of the width of the strip) eliminates, as we already said, this difficulty.

Two-dimensional paper electrophoresis of human plasma fraction I (according to COHN *et al.*). *X* = origin. *K* = direction in the first phase. Potential, 300 V. Duration, 360 min. Phosphate buffer, pH 8·0, ionic strength 0·01. *Y* = direction in the second phase. Potential, 450 V. Duration, 600 min. Phosphate buffer, pH 5·7, ionic strength 0·1. – From the left descending to the right: gamma-globulins, fibrinogen, beta- and alpha-globulins, albumin.

In our work we have always carried out two-dimensional electrophoresis on the horizontal plane and we have checked that the path taken is due almost exclusively to the migration of the ions under the difference of potential and to the electroendosmotic flow (the effects of this latter are proportionately reduced if MUNKTELL paper 20/150 is used). Working in the two phases of the process, with the same buffer (same pH and same ionic strength) the ratio between the speed of the individual components remains constant with variations of the difference of the potential applied and, at the end of the second phase, the various substances are found along a straight line, the position of which on the plane depends solely on the characteristics and duration of the passage of the current. Each individual component is found at the extremity of the hypotenuse of a right angled triangle, the other sides of which represent the path taken by the substance in the two different phases of the process.

[1] A section of this paper was read at the XIII International Congress of Pure and Applied Chemistry, Stockholm ,1953.

[2] M. MACHEBOEUF et al., Bull. Soc. Chim. Biol. *35*, 334, 346 (1953).

[3] H. H. STRAIN and J. C. SULLIVAN, Anal. Chem. *23*, 816 (1951). – F. MICHEEL and F. P. VAN DE KAMP, Angew. Chem. *64*, 607 (1952). – R. CONSDEN and W. M. STAINER, Biochem. J. *15*, xix, (1952); Nature (London) *169*, 783 (1952).

[4] H. SVENNSON and J. BRATTSTEN, Ark. Kemi *1*, 401 (1949). – W. GRASSMANN and K. HANNIG, Naturwissenschaften *37*, 397 (1950); Angew. Chem. *62*, 170 (1950). – E. L. DURRUM, J. Amer. Chem. Soc. *73*, 4875 (1951). – T. R. SATO, W. P. NORRIS, and H. H. STRAIN, Anal. Chem. *24*, 776 (1952).

[5] E. L. DURRUM, J. Colloid Sci. *6*, 274 (1951).

[6] G. DICASTRO, J. Polymer Sci. (in press).

[7] H. G. KUNKEL and A. TISELIUS, J. Gen. Physiol. *35*, 89 (1951).

Two-dimensional electrophoresis is particularly useful for discovering whether or not the tail ends or the spreading out of the spots are due to extraneous electric factors.

The use of the small siphons described before also enables the pH of the buffer to be changed between one phase and another of the electrophoresis, without drying the sheet and without causing the substance to move; even simple drying in the air can cause alteration in the proteins. Usually we preferred not to change the salts of the buffer, since (as for free electrophoresis) this can cause alterations of the mobility, independent of the variation in pH.

Usually we carried out the first phase of the electrophoresis with a buffer having a weak ionic strength, and the second phase with a buffer of higher ionic strength. The salts of the buffer were the same but in different proportions and, therefore, the pH was different. In the interval between the two phases, the sheet of paper (always kept between the two glass plates) was raised so that the siphons no longer dipped into buffer, and then was lowered again (turned 90 degrees) only when the buffer had been changed.

The sheets of paper that we used (Munktell paper 20/150 in squares of 30·5 cm each side) retained under our experimental conditions about 35 ml of solutions of electrolyte; when the paper was placed in contact with the new buffer, it reached a pH of equilibrium within 10–15 min and this pH was practically equal to that of the second buffer.

This ability to vary, between one phase and the other, not only the characteristics of the current applied but also, through the pH, the mobility of the substances, has enabled the most diversified mixtures to be studied.

We have found that this is very useful in studying plasmatic fractions. In separating fraction I, according to method 6 of Cohn and his colleagues[1], after a first electrophoresis at pH 8·0 in phosphate buffer, during the second phase we worked with the same buffer with a pH corresponding to the isoelectric point of the fibrinogen, that is, of the principle component of this fraction. Even if there was a mobility decrease for all the other components, the fibrinogen remained at the point reached at the end of the first phase, and if the second period of the electrophoresis lasted a sufficiently long time, it was possible to obtain a clear cut separation, which was very useful from a quantitative point of view.

One should bear in mind, however, that the isoelectric points noted are always slightly higher than those found in free electrophoresis[2], and perhaps this is due to the influence of the electroendosmosis. In any case, we have always preferred to work under the conditions described rather than try to overcome this influence, raising the level of the buffer in the cathode compartment. For example, an isoelectric point at pH 5·68–5·73 was observed for fibrinogen in phosphate buffer of 0·1 ionic strength in comparison with that of 5·53 checked in free electrophoresis.

G. Dicastro

*«Medital» Laboratories Research Division, Rome, July 28, 1953.*

*Riassunto*

Per una buona esecuzione dell'elettroforesi su carta è necessario lavorare con alcune precauzioni, che rendono i risultati indipendenti dalla posizione iniziale delle sostanze. In tal modo è anche possibile eseguire l'elettroforesi bidimensionalmente, con cambiamento del pH e della forza ionica del tampone tra una fase e l'altra, modificando così le velocità di migrazione.

[1] E. J. Cohn et al., J. Amer. Chem. Soc. *68*, 459 (1946).
[2] V. Schwarz, Nature (London) *167*, 404 (1951).

## Phosphorylation Mechanisms in Cloudy Swelling

Previous papers have shown that the purest form of experimental cloudy swelling is obtained when animals have been intoxicated with bacterial toxins[1]. The mild morphological changes which occur in such a cellular metamorphosis are constantly accompanied by chemical and enzymatic alterations, e.g. the protein-N is increased[2], DNA content is lowered in cells of the renal cortical tubules[3], the alkaline phosphatase distribution in the kidney is modified[4] and its activity markedly decreased[5]. In renal cells oxygen uptake and fatty acid oxidase activity are depressed by cloudy swelling[6]. Moreover, it has been observed that cyclophorase preparations from kidney, brain, heart and skeletal muscle of diphtheria intoxicated guinea-pigs are inhibited in their capacity to catalyze the processes of the tricarboxylic acid cycle in the later stages of the intoxication[7].

It has been known since long that mitochondrial changes occur in cloudy swelling, and recently Zollinger[8] added new and interesting data about it. Furthermore, it has been pointed out that heart-mitochondria, caused to swell by changing the electrolyte concentration of the medium, show a lowered cyclophorase activity[9] and a decreased P:O ratio[10].

Recent research has shown that in diphtheria intoxicated rabbits, whose organs are notoriously affected by cloudy swelling, the glucose charge does not produce the lowering of inorganic phosphate concentration in blood so much as in the normal control[11]. Hypophosphataemia occurring in normal animals after glucose injection is due to an increased phosphate uptake in tissues, i.e. it depends upon the phosphorylation of the introduced glucose.

It therefore appeared reasonable to assume that in the diphtheria intoxicated animals the functional activity of the phosphorylating systems could be decreased.

In order to clarify the mechanisms involved, the hexokinase activity and the acid-soluble phosphorus fractions have been determined in guinea-pig tissues, both normal and affected by cloudy swelling. The phosphorus fractions have been studied, both in the controls and in the experimental animals, either fasting or after glucose charge.

[1] A. Fonnesu, Boll. Soc. ital. Biol. sper. *28*, 482 (1952); Arch. Sci. biol. (in press). – A. Fonnesu and C. Severi, Brit. J. exp. Path. *34*, 341 (1953); Riv. Biol. *44*, 381 (1952).
[2] G. Popják, J. Path. Bact. *60*, 75 (1948).
[3] A. Fonnesu and C. Severi, Brit. J. exp. Path. *34*, 341 (1953).
[4] A. Fonnesu and C. Severi, Riv. Biol. *44*, 381 (1952).
[5] C. Severi, Boll. Soc. ital. Biol. sper. *28*, 1925 (1952).
[6] A. Fonnesu, Boll. Soc. ital. Biol. sper. *28*, 482 (1952); Arch. Sci. biol. (in press).
[7] O. Hoffmann-Ostenhof, O. F. Schwarz, W. Zischka, and H. Eibl, IIe Congr. int. Biochimie, Paris; Communications 35 (1952).
[8] H. U. Zollinger, Exper. *4*, 312 (1948); *6*, 14 (1950); Rev. Hématol. *5*, 696 (1950).
[9] J. W. Harman, Amer. J. Pathol. *26*, 687 (1950); Expt. Cell. Res. *1*, 394 (1950).
[10] J. W. Harman and M. Feigelson, Expt. Cell. Res. *3*, 509 (1952).
[11] A. Fonnesu and C. Severi, Boll. Soc. ital. Biol. sper. *28*, 1923 (1952).

Cloudy swelling in guinea-pig tissues was produced experimentally by subcutaneous injection of diphtheria toxin (1 M.L.D./250 g of body weight). Liver, kidney, heart and skeletal muscle were taken 24 h after the toxin injection.

Hexokinase activity was estimated in tissue homogenates according to Long[1].

Fractionation of acid-soluble phosphorus was carried out as described by Pinchot and Bloom[2] and phosphorus concentration in the fractions determined by the method of Fiske and Subbarow[3].

*Table I*

Hexokinase activity of tissues of guinea-pig injected with diphtheria toxin compared with normal controls

| Tissue | $-Q_{glucose}$[1] | |
|---|---|---|
| | Normal guinea-pig | Diphtheria intoxicated guinea-pig |
| | [2] | [2] |
| Kidney (whole) . . | *10,0* 9,1–10,6 (3) | *10,1* 9,6–10,6 (3) |
| Heart . . . . . | *8,6* 6,9–9,6 (3) | *8,4* 6,3–9,8 (3) |
| Skeletal muscle . . | *5,4* 4,4–6,6 (3) | *5,3* 3,9–6,2 (3) |
| Liver . . . . . | *1,6* 1,4–1,9 (3) | *1,8* 1,4–2,0 (3) |

[1] $-Q_{glucose}$ = $\mu$l glucose disappearing/mg dry weight/h, where 180 $\mu$g glucose = 22,4 $\mu$l.

[2] Mean, range and number of observations (in parentheses).

The glucose charge was performed with intracardiac injection of 0·2 g of glucose/100 g of body weight. Tissues employed for fractionation of acid-soluble phosphorus were removed from animals under $MgSO_4$ anaesthesia; when animals were given glucose, anaesthesia began at a time corresponding to the lowest level of inorganic phosphate concentration in the blood (15 min).

[1] C. Long, Biochem. J. *50*, 407 (1951).

[2] G. B. Pinchot and W. L. Bloom, J. biol. Chem. *184*, 9 (1950).

[3] C. H. Fiske and Y. Subbarow, J. biol. Chem. *66*, 375 (1925).

The results of the hexokinase activity measurements are summarized in Table I; the data show clearly that the enzymatic activity is unchanged in tissues affected by cloudy swelling, as compared with controls.

The results of the phosphorus determinations in normal animals and in animals injected with diphtheria toxin, both examined fasting and after glucose charge, cannot be reported here extensively and are briefly summarized in Table II.

The following fractions have been considered: 180 min P, ester P, labile P (ATP, ADP), phosphocreatine P (PCP) and total high energy bond phosphorus (total ~P). All the fractions have been studied in liver, kidney, heart and skeletal muscle.

The mean values of the phosphorus determinations, carried out in tissues of fasting diphtheria intoxicated guinea-pigs, show, as compared with those of the relative controls, a statistically significant increase of inorganic P in liver and a significant decrease of phosphocreatine P, with a comparable rise of inorganic P, in muscle. A comparable fall of total ~P takes place since ATP is unchanged. It should be noted here that our findings for muscle are well in agreement with the data found by Pinchot and Bloom[1].

No significative change of the phosphorus-content in the fractions is induced in tissues of normal guinea-pigs by the injection of glucose. On the contrary, the glucose-charge in guinea-pigs treated with diphtheria toxin causes, in kidney and in muscle, a significant increase of inorganic P with a fall of ATP and of total ~P. No further lowering of phosphocreatine P, already decreased in the fasting intoxicated animals, is induced by glucose injection in diphtheria intoxicated guinea-pigs.

The evidence presented shows, therefore, that in cloudy swelling a marked fall in total high energy bond phosphorus takes place. This is particularly evident after glucose charge because the lowering involves both phosphocreatine and ATP.

Although a significant decrease of total ~P is observed only in certain tissues and chiefly in muscle, yet it appears probable that the phenomenon may also occur in other tissues, where the ~P content is too small to appreciate the change.

From the results referred in the present paper it seems quite reasonable to conclude that in tissues affected by cloudy swelling the formation of ~P is markedly reduced.

[1] G. B. Pinchot and W. L. Bloom, J. biol. Chem. *184*, 9 (1950).

*Table II*

Acid-soluble phosphorus fractions in organs of diphtheria intoxicated guinea-pigs either fasting or after glucose charge
Variations per cent from the control values[1]

| Fraction | Diphtheria intoxicated guinea-pig | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fasting | | | | After glucose charge | | | | | | | |
| | Liver | Kidney | Heart | Muscle | Liver | | Kidney | | Heart | | Muscle | |
| | [2] | [2] | [2] | [2] | [3] | [4] | [3] | [4] | [3] | [4] | [3] | [4] |
| 180 min P. . . . . | — | — | — | — | — | — | — | — | — | — | — | — |
| Ester P . . . . . | — | — | — | — | — | — | — | — | — | — | — | — |
| Labile P (ATP, ADP). . . . . | — | — | — | — | — | — | — | −60% | — | — | −42% | −47% |
| PCP . . . . . | — | — | — | −52% | — | — | — | — | — | — | −37% | — |
| Inorganic P . . . . . | +34% | — | — | +27% | +59% | — | +20% | — | — | — | +57% | +24% |
| Total ~P. . . . . | — | — | — | −16% | — | — | — | −51% | — | — | −40% | −34% |

[1] Only the changes statistically significant are reported (mean values).

[2] Relative to the normal fasting guinea-pig.

[3] Relative to the normal guinea-pig receiving glucose.

[4] Relative to the fasting diphtheria intoxicated guinea-pig.

Such a conclusion offers a satisfactory explanation of the disturbance observed in diphtheria intoxicated animals, i.e. the reduced hypophosphataemia after glucose charge in spite of the normal hexokinase activity.

Moreover, since the formation of ~P occurs chiefly in the mitochondria, it appears very probable that the metabolic alteration may be in relation to the known mitochondrial changes observed in cells in cloudy swelling.

A. Fonnesu and Clara Severi

*Institute of General Pathology, University of Perugia, Italy, February 25, 1953.*

*Riassunto*

Negli organi in rigonfiamento torbido sperimentale da tossina difterica l'attività esocinasica è immodificata, mentre è rallentata la formazione di ~P. Ciò spiega perchè, negli animali intossicati con tossina difterica, il carico di glicoso determina ipofosfatemia meno netta. La diminuita formazione di ~P viene messa in rapporto con le alterazioni mitocondriali osservate nelle cellule in rigonfiamento torbido.

## Essais d'inhibition de l'action inflammatoire cutanée du chloroforme chez le rat

L'application du chloroforme sur la peau du lapin provoque à l'endroit irrité l'apparition des colorants vitaux préalablement injectés par voie intraveineuse. Ambrose et de Eds ont donné au phénomène une expression quantitative fonction de sa période latente[1]. Dans une note antérieure[2], nous avons signalé l'influence de la cortisone, de l'A.C.T.H. et des salicylés sur le délai d'apparition des colorants au niveau de la peau irritée conformément à la technique originale de ces auteurs. Aujourd'hui, nous adapterons au rat le test d'Ambrose et de Eds et étudierons ses variations sous l'influence d'agents hormonaux ou pharmacodynamiques doués, selon la littérature, de propriétés antiinflammatoires. Nous rechercherons aussi chez cet animal l'influence de la surrénalectomie sur l'apparition du colorant au niveau des téguments irrités par le chloroforme.

*Technique.* Nous utilisons des rats adultes albinos, d'un poids moyen de 150 g provenant de deux élevages différents. Aucune différence entre les lots n'a été constatée.

1° La peau du dos est rasée la veille de l'expérience. Le lendemain, les rats reçoivent, par voie intrapéritonéale, 1 ml d'une solution de chlorazol blue sky, à 1% dans du sérum physiologique. Trois heures après, les animaux sont fixés sur le ventre. Un tampon de papier filtre de 2 cm² environ, imbibé de chloroforme et rapidement essoré, est appliqué fermement sur la peau durant 30 s. Un chronomètre est alors mis en marche et le temps minimum nécessaire pour qu'apparaissent les premières traces de coloration bleuâtre au niveau du derme est noté.

Deux tests sont pratiqués sur le même animal, de façon symétrique par rapport à la colonne vertébrale.

2° Les substances suivantes ont été administrées avant de pratiquer le test au chloroforme.

*A.C.T.H:* 1 mg/100 g de poids du corps par voie intramusculaire deux fois par jour pendant 48 h.

*Extrait cortical total:* 0,25 ml de Cortine Organon (1 ml = 50 g de glande fraiche), une fois par jour, durant 48 h.

*Cortisone:* 1 mg/100 g de poids du corps, deux fois par jour, pendant 48 h, par voie intramusculaire.

*Salicylate de soude:* 300 mg/kg de poids du corps, deux fois par jour par voie intrapéritonéale, pendant 48 h.

Pour ces quatre produits, la dernière injection a lieu au moment de l'injection intrapéritonéale du colorant.

*Phénergan:* 5 mg par voie intramusculaire et 5 mg par voie souscutanée lors de l'injection du bleu.

3° La surrénalectomie a été pratiquée par voie dorsolombaire sous courte anesthésie à l'éther, la veille de l'administration du bleu. Certains animaux surrénalectomisés ont reçu de l'extrait cortical total à raison de 0,5 ml de Cortine Organon au moment de l'intervention. Chez d'autres qui ont reçu la même dose de Cortine au moment de la surrénalectomie, 300 mg/kg de salicylate de soude ont été injectés par voie intrapéritonéale au moment de l'administration du colorant vital.

*Résultats.* 1° Les tableaux I et II mentionnent, exprimés en secondes, les délais moyens d'apparition de la coloration bleuâtre au niveau du derme irrité.

2° La surrénalectomie entraîne une mortalité importante des animaux injectés de bleu, plus encore des animaux injectés simultanément de bleu et de salicylate de soude. La Cortine prolonge nettement la survie des animaux injectés.

[1] A. Ambrose et F. de Eds, J. Exp. Pharmacol. Therap. *90*, 359 (1947).

[2] H. Van Cauwenberge et J. Lecomte, Exp. *8*, 469 (1952).

*Tableau I*

| Lots | Nombre d'essais | Délai moyen en secondes | $\sigma$ de la moyenne* | $\theta$ coefficient de Bernoulli• |
|---|---|---|---|---|
| Témoins . . . | 48 | 456 | 201 | |
| A.C.T.H. . . . | 68 | 253 | 84 | 0,99 |
| Cortine . . . . | 34 | 306 | 107 | 0,69 |
| Cortisone . . . | 36 | 340 | 179 | 0,46 |
| Salicylate . . . | 16 | 678 | 218 | 0,79 |
| | 16 | >7200 | — | — |
| Phénergan . . | 27 | 1197 | 770 | 0,93 |

*Tableau II*

| Lots | Opérés | Décès | Nombres d'essais | Délai moyen en secondes | $\sigma$ de la moyenne* | $\theta$ coefficient de Bernoulli • |
|---|---|---|---|---|---|---|
| Témoins . . . . . | 0 | 0 | 48 | 456 | 201 | |
| Surrénalectomie . . . . . | 22 | 15 | 14 | >7200 | — | — |
| Surrénalectomie + Cortine . . . . . | 14 | 2 | 24 | 1296 | 422 | 1,81 |
| Surrénalectomie + Cortine + Salicylate | 12 | 6 | 12 | 1853 | 363 | 1,97 |

* $\sigma$: écart statistique: $\sqrt{\sum (x-\bar{x})^2/n}$.  • $\theta$: coefficient de probabilité: $X^1-\bar{X}/\sqrt{\sigma_1^2+\sigma_2^2}$.

L'influence de la surrénalectomie figure dans le Tableau II.

*Discussion.* 1° Les hormones corticotropes et surrénaliennes n'ont aucune influence inhibitrice sur le délai d'apparition du bleu. La fixation de ce colorant paraît même meilleure lorsque l'animal a été traité par Cortine, cortisone ou A.C.T.H.

Le Phénergan allonge le temps d'apparition. Il est impossible de décider s'il s'agit d'une neutralisation des effets de l'histamine cutanée libérée par le chloroforme. L'administration dermique d'histamine provoque en effet la fixation locale du bleu. Le salicylate de soude est l'agent qui retarde le plus le passage extravasculaire du colorant, même, semble-t-il, après surrénalectomie.

2° Les résultats que nous a donnés l'A.C.T.H. chez le rat sont en discordance avec ceux notés antérieurement chez le lapin. En effet, les valeurs observées chez ce dernier animal injecté de l'hormone corticotrope pour le délai d'apparition du bleu se divisent en deux groupes: des valeurs voisines de la normale d'une part, des valeurs fortement prolongées d'autre part (Van Cauwenberge et Lecomte, *loc. cit.*).

Nous n'avons observé chez les rats traités par A.C.T.H. que des valeurs voisines de la normale. Par contre, l'emploi du salicylate de soude divise, chez le rat comme chez le lapin, les animaux en deux groupes: l'un où les chiffres relevés sont modérément augmentés par rapport aux témoins, l'autre où la coloration n'apparaît pas dans la peau irritée après deux heures et plus, bien que le bleu soit passé dans le sang de l'animal.

Ces faits sont en accord avec les résultats de nos essais chez le rat surrénalectomisé, injecté ou non de salicylate de soude par voie intrapéritonéale. Ils constituent, pensons-nous, la première démonstration certaine d'une action *in vivo* du salicylate de soude qui ne soit pas déterminée par une activation du système hypophyso-surrénalien.

3° La surrénalectomie fait disparaître toute fixation locale du bleu. L'administration de Cortine à fortes doses corrige partiellement les effets de l'ablation des glandes surrénales. La fixation du bleu paraît donc sous la dépendance des hormones cortico-surrénaliennes.

H. van Cauwenberge
J. Lecomte et J. Goblet

*Institut de clinique et de pathologie médicales de l'Université et Fonds national de la Recherche scientifique, Liège, le 3 juillet 1953.*

*Summary*

(1)—In the rat, total extracts of cortex, cortisone and A.C.T.H. do not retard the fixation of a vital colorant at the level of a cutaneous zone irritated by chloroform.

(2)—Phenergan prolongs the delay in the appearance of the blue.

(3)—Sodium salicylate selectively inhibits the inflammatory cutaneous action of chloroform, even after adrenalectomy.

## Interrelationship between Vitamin $B_{12}$ and Pantothenic Acid in the Metabolism of "Wild" Strains of *Escherichia coli*

The absorption of vitamin $B_{12}$ by cells of *Escherichia coli* was demonstrated in 1951 by Burkholder[1]. The importance of this phenomenon in the etiology of Addisonian pernicious anemia was emphasized by Burkholder[1] and by Hoff-Jörgensen[2] and his coworkers in 1952. The effect of this absorption on the ability of *Escherichia coli* to synthesize other members of the vitamin B group has not yet been studied.

The author's purpose in carrying out this work was to study the effect of vitamin $B_{12}$ on the biosynthesis of other vitamins B in "wild" strains of *E. coli* because of the importance of this disturbance of the equilibrium between B-group vitamins, especially in the intestinal synthesis of vitamins.

In these tests the medium described by Burkholder[3] for the microbiological determination of vitamin $B_{12}$ was used as nutrient fluid. In the first tests the following modification of this medium was used:

| | |
|---|---|
| Ammonium tartrate . . . . . | 7 g |
| Ammonium sulphate . . . . . | 3 g |
| Dibasic potassium phosphate . . . | 2 g |
| Lactose . . . . . | 5 g |
| Vitamin-free Casamino acids (Difco) | 1 g |
| Aqua dest. ad 1000 ml, pH adjusted to | 6·5 |

250 ml of this medium was pipetted into three Erlenmeyer bottles and sterilised. Vitamin $B_{12}$ was added to the bottles as follows:

| | |
|---|---|
| Bottle No. 1, Vitamin $B_{12}$. . . | 0·0 $\gamma$ |
| Bottle No. 2, Vitamin $B_{12}$. . . | 7·5 $\gamma$ |
| Bottle No. 3, Vitamin $B_{12}$. . . | 15·0 $\gamma$ |

All the bottles were inoculated with one drop of a fresh culture of a "wild" strain of *E. coli*, isolated from human faeces. After 72 h incubation at 37°C, the living cells were harvested with a centrifuge at 5000 r.p.m.

From the clear supernatant fluid the following members of vitamin B group were determined: Nicotinic acid, pantothenic acid, folic acid and biotin. The methods published in 1951 by the Association of Vitamin Chemist's were used in all the assays.

Folic acid was estimated using *Streptococcus faecalis* as test organism, while the other vitamins were assayed with *Lactobacillus arabinosus* 17–5 (Table I).

The addition of 2 g of asparagine, 0·2 g of sodium citrate, 0·1 g of potassium chloride, 0·2 g of magnesium sulphate and 50 mg of *dl*-tryptophane, and the substitution of the lactose of the medium by glucose could not effect the synthesis of pantothenic acid when this strain of *E. coli* was used in the experiments.

The synthesis of the above-mentioned members of the vitamin B group was not influenced by the addition of vitamin $B_{12}$ to the growth media. The synthesis of pantothenic acid was first discovered in a medium in which the growth conditions for *E. coli* were considerably better. The medium used by the author was that proposed in 1951 by Burkholder[3] as mentioned above.

In the first tests, 20 ml of this medium was pipetted into four test tubes. The addition of vitamin $B_{12}$ was as follows: Tube No. 1, 0·0 $\gamma$ : Tube No. 2, 0·5 $\gamma$ : Tube No. 3, 1·0 $\gamma$ : Tube No. 4, 1·5 $\gamma$.

The test proceeded as above and after 72 h' growth the cells were centrifugated off and the pantothenic acid content of the supernatant fluid was determined.

[1] P. Burkholder, Science *114*, 478 (1951).

[1] P. Burkholder, Arch. Biochem. Biophysic. *39*, 322 (1952).

[2] E. Hoff-Jörgensen, A. P. Skouby, and J. Gad Andersen, Nord. Med. *48*, 1754 (1952).

[3] P. Burkholder, Science *114*, 459 (1951).

*Table I* Results

| | Vitamin $B_{12}$ in $\gamma$ per ml of medium | Synthesized vitamins in $\gamma$ per ml of medium | | | |
|---|---|---|---|---|---|
| | | Nicotinic acid | Pantothenic acid | Folic acid | Biotin |
| Bottle No. 1 . . . | 0·00 | 0·06 | none | 0·005 | 0·0008 |
| Bottle No. 2 . . . | 0·03 | 0·06 | none | 0·005 | 0·0008 |
| Bottle No. 3 . . . | 0·06 | 0·05 | none | 0·005 | 0·0006 |

A second test was made with the same strain of *E. coli* and with the same growth medium. Another strain of *E. coli*, also isolated from human faeces, was used in this experiment. It was observed that the

*Table II* Results

| | Vitamin $B_{12}$ in $\gamma$ per ml of medium | Synthesized pantothenic acid in $\gamma$ per ml of medium |
|---|---|---|
| Tube No. 1 . . | 0·00 | 0·06 |
| Tube No. 2 . . | 0·025 | 0·03 |
| Tube No. 3 . . | 0·05 | none |
| Tube No. 4 . . | 0·075 | none |

ability of this strain to synthesize pantothenic acid was considerably greater than that of strain No. 1. Before centrifugation the turbidity of the suspensions was measured with a Pulfrich photometer.

*Table III* Results

| | Vitamin $B_{12}$ in $\gamma$ per ml of medium | Turbidity of the suspensions in 0·5 cm cuvette | Synthesized pantothenic acid in $\gamma$ per ml of medium |
|---|---|---|---|
| Strain No. 1 | 0·00 | 0·62 | 0·01 |
| | 0·03 | 0·59 | 0·007 |
| | 0·06 | 0·63 | none |
| Strain No. 2 | 0·00 | 0·66 | 0·1 |
| | 0·03 | 0·62 | 0·08 |
| | 0·06 | 0·61 | none |

The results show a very clear inhibition in the synthesis of pantothenic acid caused by the addition of vitamin $B_{12}$.

A third strain of *E. coli* was also tested in this respect. The results in this test show a clear decrease in the amount of synthesized pantothenic acid, although the

*Table IV* Results

| Vitamin $B_{12}$ in $\gamma$ per ml of medium | Turbidity in 0·5 cm cuvette | Synthesized pantothenic acid in $\gamma$ per ml of medium |
|---|---|---|
| 0·00 | 0·82 | 0·07 |
| 0·03 | 0·82 | 0·06 |
| 0·06 | 0·82 | 0·04 |

The turbidity of 0·82 observed in all the suspensions corresponded to $3{\cdot}2 \times 10^9$ cells per ml.

effect of added vitamin $B_{12}$ is not so dramatic as in earlier series.

To avoid the influence of the nutrient medium, the following test with strain No. 1 was also conducted. The growth medium for *E. coli* used in these experiments contained the following substances:

| | |
|---|---|
| Vitamin-free Casamino acids (Difco) . | 2 g |
| Peptone (Difco) . . . . . | 20 g |
| Lactose . . . . . | 10 g |
| Sodium chloride . . . . . | 0·5 g |
| Aqua dest. ad 1000 ml, pH adjusted to | 6·3 |

After sterilisation, an inoculation with cells of strain No. 1 was made. After 72 h' growth the cells were harvested with a Sharpless supercentrifuge and washed with saline solution. The washed cells were suspended in saline solution. This suspension contained $4{\cdot}10^9$ cells per ml. 6 ml of this suspension was pipetted aseptically into each of two sterilized centrifuge tubes. To one of the tubes 0·5 $\gamma$ vitamin $B_{12}$ on 0·5 ml saline solution was added under aseptic conditions, and to the other tube a corresponding amount of saline. The tubes were incubated for 12 h at 37°C, after which the pantothenic acid content of the tubes was determined.

*Table V* Results

| | Vitamin $B_{12}$ in the tube | Pantothenic acid in the tube |
|---|---|---|
| Tube No. 1 . . . | 0·00 $\gamma$ | 0·42 $\gamma$ |
| Tube No. 2 . . . | 0·5 $\gamma$ | 0·24 $\gamma$ |

The results in this series verify the earlier observations in this study.

In all the tests the growth of *E. coli* was approximately the same in the corresponding test series. However, the synthesis of pantothenic acid was very clearly decreased or completely blocked by the addition of vitamin $B_{12}$ to the media. The synthesis of other investigated members of the vitamin B group, i.e. nicotinic acid, folic acid and biotin, was not influenced in any way by the addition of vitamin $B_{12}$. It was observed that the same concentration of vitamin $B_{12}$ effected an unequal inhibition of pantothenic acid synthesis in the different strains. Further it was noted that the inhibition of pantothenic acid synthesis was greatest when the used strains were fresh and had not been kept very long on stab agar cultures.

In this connection it is noteworthy that Evans[1] and his co-workers, Yacowitz[2] and his coworkers, and

[1] R. J. Evans, A. C. Groschke, and H. A. Butts, Arch. Biochem. Biophys. *31*, 454 (1951).

[2] H. Yacowitz, L. C. Norris, and G. F. Heuser, J. Biol. Chem. *192*, 141 (1951). – *Vitamin Methods* (Interscience Publishers, New York, 1951), p. 180, 205, 231, 243.

Welch and Couch[1] observed in 1951 an interrelationship between vitamin $B_{12}$ and pantothenic acid in chicken liver cells. The nature of this phenomenon is still unexplained.

The phenomenon observed by the author has also no explanation, but because of its biological importance it deserves more investigation.

J. Jännes

*Department of Medical Chemistry, University of Helsinki, July 4, 1953.*

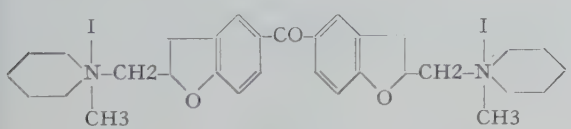
*Zusammenfassung*

Drei «wilde» Stämme von *Escherichia coli* wurden auf vitaminfreien Nährböden gezüchtet. Zusatz von Vitamin $B_{12}$ in Konzentrationen von 0,03 bis 0,06 $\gamma$ je ml ergab bei verschiedenen Stämmen eine ungleiche Hemmung der Pantothensäure-Synthese. Die eindeutigsten Resultate wurden mit frischen Stämmen erzielt, die nur kurz auf Agar gezüchtet wurden.

[1] B. E. Welch and J. R. Couch, Abstracts Amer. Chem. Soc. Southwest Regional Meeting 35–36 (Austin,Texas, Dec. 1951), cited by A. D. Welch, and C. A. Nichol in Ann. Rev. Biochem. *1952* Stanford, Calif. p. 674.

## Sur l'inhibition sélective des acétylcholinestérases *in vivo* chez le chien

Hawkins et Mendel[1] ont récemment décrit un agent anticholinestérasique, le N-p-chlorophényl-N-méthylcarbamate de m-hydroxy-phényl-triméthylammonium (Nu-1250) grâce auquel ils ont, chez le rat, inactivé les acétylcholinestérases (AcChE) plasmatiques sans inhiber les cholinestérases non spécifiques de l'acétylcholine (XChE); dans ces conditions ils ont observé l'apparition de différents symptomes acétylcholinomimétiques et ils ont conclu, de ce fait, que les XChE n'ont aucun rôle, même auxiliaire, dans l'hydrolyse *in vivo* de l'acétylcholine. Il eût convenu, pour étayer cette conclusion, de réaliser des expériences similaires chez des animaux se prêtant à des analyses pharmacologiques plus détaillées, mais le Nu-1250 s'avéra, chez le chien, dépourvu d'activité antiacétylcholinestérasique sélective (Casier et de Vleeschhouwer[2]).




3318 CT

[1] R. D. Hawkins et B. Mendel, Biochem. J. *44*, 260 (1949).

[2] H. Casier et G. R. de Vleeschhouwer, Arch. int. Pharmacodyn. *90*, 412 (1952).

La synthèse, par Funke et von Däniken[1], d'un nouvel antiacétylcholinestérasique[2], le di-iodométhylate de la bis(pipéridinométhylcoumaranyl-5) cétone (3318 CT), nous a permis d'entreprendre une étude de l'inhibition préférentielle des AcChE chez le chien, et nous résumons ici les résultats observés jusqu'à présent.

### *I.–Activités anticholinestérasiques du 3318 CT*

*a*) *Essais in vitro* (Tableau I). Les expériences ont été réalisées avec la méthode de Warburg-Ammon[3], et cela dans des conditions classiques, en utilisant l'acétylcholine elle-même comme substrat. – Comme le Nu-1250, le 3318 CT inhibe l'AcChE globulaire de l'homme pour des concentrations environ 10 000 fois inférieures à celles nécessaires pour inactiver la ChE plasmatique dans les mêmes proportions. – En outre, et contrairement au Nu-1250, le 3318 CT est aussi un antiacétylcholinestérasique sélectif dans le cas des enzymes du chien, le rapport activité anti-AcChE : activité anti-ChE étant ici de 10 000 à 100 000; cette activité antiacétyl-cholinestérasique est très élevée, environ 10 fois supérieure à celle du Nu-1250 sur l'AcChE globulaire humaine et de 10 à 100 fois supérieure à celle de la Prostigmine sur l'AcChE globulaire du chien. Comme la prostigmine et comme le Nu-1250, le 3318 CT est un inhibiteur réversible car l'inhibition observée rétrocède lorsque le complexe enzyme-substrat est dilué.

*b*) *Essais in vivo*. L'existence d'un effet de dilution nous a conduit à réaliser la majorité de nos déterminations (Tableau II) avec du sang (ou du plasma) aussi peu dilué que possible et avec des substrats dits spécifiques, c'est-à-dire avec de la benzoylcholine (0,01 *M*) pour les activités des XChE, et de l'acétyl-$\beta$-méthylcholine (0,03 *M*) pour celles des AcChE. En plus des corrections classiques, ceci a nécessité la détermination des coefficients de rétention du sang (ou du plasma)[4]. Le sang était systématiquement prélevé avant et une demi-heure après l'injection de 3318 CT. Dans ces conditions, aucune inhibition significative des XChE sanguines ne fut observée, même après 1 mg/kg i.v., alors qu'une inactivation nette des AcChE apparut dès après 0,01 mg/kg. Des déterminations réalisées dans d'autres conditions, avec l'acétylcholine comme substrat, ont confirmé, dans les limites des erreurs qu'elles impliquent, le caractère sélectif de l'inhibition des AcChE par le 3318 CT.

[1] A. Funke et K. von Däniken, Bull. Soc. Chim. France, 457 (1953).

[2] A. Funke, J. Jacob et K. von Däniken, C. r. Acad. Sci. *236*, 149 (1953).

[3] R. Ammon, Pflügers Arch. *38*, 729 (1934).

[4] W. W. Umbreit, R. H. Burris et J. F. Stauffer, *Manometric Techniques* (Burgess Publ. Co., Minneapolis 1945), p. 115.

*Tableau I*

Inhibitions, *in vitro* (en %), de cholinestérases sanguines par le 3318 CT

| Source d'enzyme | | | Concentration du substrat (AcCh) | Concentrations de 3318 CT (M) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ |
| *Homme* | Plasma | (1/30). . . | 0,06 *M* | 82 | 45 | 10 | 5 | 5 | 7 | 2 |
| | Globules | (1/60). . . | 0,005 *M* | — | — | — | 97 | 80 | 56 | 32 |
| *Chien* | Plasma | (1/30). . . | 0,06 *M* | 43 | 9 | 3 | — | 0 | 0 | 0 |
| | Globules | (1/24). . . | 0,005 *M* | — | 95 | 94 | 84 | 60 | 34 | — |

*Tableau II.* Inhibitions, *in vivo*, des cholinestérases du sang de chien par le 3318 CT

| Chien N° | 3318 CT injection i.v. (mg/kg) | Source d'enzyme (concentration terminale: = 0,75) | Inhibition (en %) des XChE (substrat: benz. Ch : 0,01 *M*) | Inhibition (en %) des AcChE (substrat: Ac. -méthyl-Ch. 0,03 *M*)** |
|---|---|---|---|---|
| 1 | 0,001 | sang total | — | 0 |
| 1 | 0,01 | sang total | — | 20 |
| 1 | 0,10 | sang total | — | 65 |
| 2 | 0,10 | sang total | 3 | 60 |
| 3 | 0,20 | plasma séparé | 0 | 65 |
| 4 | 0,25 | sang total | 8 | 70 |
| 5* | 0,25 | sang total | 0 | — |
| 6* | 0,40 | sang total | — | >50 |
| 7 | 1 | sang total | 8 | >50 |

* Les chiens 5* et 6* étaient éveillés, les autres animaux chloralosés et ventilés artificiellement.

** La précision de ces déterminations est faible en raison de causes d'erreurs dans le détail desquelles nous ne pouvons entrer ici.

*II.–Propriétés pharmacologiques du 3318 CT*

L'administration intraveineuse de 0,02 à 0,5 mg/kg de 3318 CT est suivie de myosis, de salivation, de lacrymation, de diarrhée, de secousses musculaires fibrillaires et fasciculaires, de parésie et de dyspnée. Le chien éveillé meurt par asphyxie, avec 0,3 à 0,5 mg/kg i.v.; le chien narcosé et ventilé artificiellement supporte des doses plus élevées; l'atropinisation prévient ou abolit les symptômes muscariniques. Dans l'ensemble, et à l'exception de la parésie motrice, les actions du 3318 CT nous ont paru moins intenses que celles provoquées par des doses égales de prostigmine; cette différence est extrême dans le cas de l'effet sur la fréquence cardiaque: chez le chien chloralosé, cette dernière n'est qu'exceptionnellement ralentie – et seulement très modérément – par le 3318 CT. Cette substance sensibilise nettement la bradycardie acétylcholinique dès après 0,5 $\mu$g par kg et la bradycardie par faradisation prolongée (1 min ou plus) du nerf vague après 5-20 $\mu$g par kg; la bradycardie par faradisation brève (5 s) et la bradycardie réflexe à l'hypertension adrénalinique sont moins nettement influencées, la bradycardie réflexe à la désocclusion carotidienne ne l'est pratiquement pas; en outre, la sensibilisation des bradycardies acétylcholinique et vagale est incomplète en ce sens que, lorsqu'elle ne progresse plus malgré l'administration répétée de 0,3 à 0,5 mg/kg de 3318 CT, il reste possible de l'accentuer considérablement par l'injection de 0,1 à 0,2 mg/kg de prostigmine. Enfin, les hypertensions acétylcholinique (chez l'animal atropinisé) et nicotinique sont accrues; il n'en est pas de même pour l'hypertension réflexe par occlusion des carotides.

En somme, le 3318 CT est capable de provoquer l'apparition de symptomes acétylcholinomimétiques et de sensibiliser divers phénomènes acétylcholiniques ou attribués à l'acétylcholine. Il existe cependant des différences nettes entre les effets du 3318 CT et ceux d'agents – comme la Prostigmine – capables, entre autres, d'inactiver à la fois les AcChE et les XChE; ces différences sont d'autant plus curieuses qu'elles traduisent surtout une activité moins étendue et moins intense pour le 3318 CT alors que son pouvoir antiacétylcholinestérasique, *in vitro*, est plus élevé que celui de la prostigmine. L'interprétation de ces différences est délicate (Koelle et Gilman[1], Heymans[2]) et il ne nous est pas possible de la discuter ici; leur seule existence nous paraît cependant montrer qu'il est prématuré de dénier aux XChE tout rôle dans la limitation des effets de la stimulation de nerfs cholinergiques.

J. Jacob

*Laboratoire de pharmacologie, Service de chimie thérapeutique de l'Institut Pasteur, Paris, le 6 juin 1953.*

*Summary*

3318 CT [bis(piperidinométhylcoumaranyl-5) cetone diméthiodide] a compound recently synthesized by A. Funke and K. von Däniken, is shown to be a potent and selective inhibitor, both *in vitro* and *in vivo*, of the blood acetyl-cholinesterase (AcChE) of the dog. It produces acetylcholinomimetic symptoms in the animal and sensitizes different responses to acetylcholine and to stimulation of cholinergic nerves without inhibiting the blood non-specific cholinesterase (XChE). However, the action of 3318 CT appears to be limited, both in nature and in intensity, when compared to that of prostigmine, although the latter substance is a less potent inhibitor of AcChE *in vitro* than is 3318 CT. This difference is probably related to many factors, one of which may be the fact that prostigmine inhibits not only AcChE but also XChE; and it is not yet ascertained that XChE plays no role at all in the limitation of the effect of the stimulations of cholinergic nerves.

[1] G. B. Koelle et A. Gilman, J. Pharmacol *95*, Part. II, p. 166 (1949).

[2] C. Heymans, *Exposés annuels de Biochimie médicale*, 12e Série (Masson & Cie, Paris 1951).

## PRO EXPERIMENTIS

### Eine Methode zur titrimetrischen Bestimmung des Eisens im Blut mit Hilfe von Komplexon

In der neuern Literatur finden sich bereits verschiedene Arbeiten über die komplexometrische Bestimmung des Kalziums im Blutserum[1], die dank ihrer bestechenden Einfachheit den klassischen Methoden weit überlegen ist. Da sich Eisen mit Komplexon nach Schwarzen-

[1] H. Flaschka und A. Holasek, Hoppe-Seylers Z. physiol. Chem. *288*, 244 (1951). – A. H. Holtz, Nederl. Tijdschr. Geneeskunde *95*, 2420 (1951). Chem. Weekblad *47*, 907 (1951). – E. Kaufmann, Schweiz. Apotheker-Z. *90*, 797 (1952).

BACH[1] ohne weiteres titrieren lässt, schien es angezeigt, auch die Möglichkeit der Anwendung dieser Methode auf die Bestimmung des Eisens im Blut zu prüfen. Wie diesbezügliche Versuche gezeigt haben, ist es möglich, die titrimetrische Bestimmung des Eisens mit Komplexon III und der Brenzkatechin-3, 5-disulfosäure («Tiron») als Indikator auf Blut zu übertragen. Da die Komplexbildung mit dem Ferriion im Gegensatz zu andern Metallionen bereits in saurem Milieu augenblicklich und quantitativ vor sich geht und zudem mit einem spezifischen Indikator gearbeitet wird, ist eine Abtrennung des Eisens von andern Kationen nicht nötig, so dass sich die Bestimmung, wie im folgenden dargelegt wird, sehr einfach gestaltet: 1,00 cm³ Blut wird in einem Mikrokjeldahl-Zerstörungskolben mit 2 cm³ konzentrierter Schwefelsäure, 2 cm³ konzentrierter Salpetersäure und 5 Tropfen konzentrierter Perchlorsäure versetzt und über freier Flamme vorsichtig erwärmt. Sobald die Hauptmenge des Wassers verdampft ist und sich die Lösung schwarz zu färben beginnt, gibt man 15 Tropfen Salpetersäure und 2 Tropfen Perchlorsäure hinzu und erwärmt weiter, bis die Stickoxyde und das Wasser entfernt sind. Die jetzt noch braune Lösung wird mit einigen Tropfen Salpetersäure aufgehellt. Man erhitzt nun stärker, wobei die Lösung hellgelb und klar wird, und raucht die Schwefelsäure möglichst vollständig ab. Die ganze Zerstörung soll nicht länger als 10 min dauern. Man lässt etwas abkühlen, gibt 10–15 Tropfen 2 *n* Natronlauge hinzu, neutralisiert weiter mit konzentrierter Lauge, bis sich ausfallendes Eisenhydroxy beim Umschwenken eben noch löst, und macht mit einigen Tropfen verdünntem NaOH schwach alkalisch. Nun erwärmt man und löst den Niederschlag mit 2 *n* Salzsäure eben wieder auf. Am Schluss soll sich ein in die Lösung, deren Volumen jetzt höchstens 2–3 cm³ beträgt, gegebenes Stückchen Kongorotpapier eben blau färben. Nun erwärmt man auf etwa 50°C, gibt eine Spatelspitze (30–50 mg) Tiron-Indikator hinzu und titriert die tief blaugrüne Lösung mit 0,01 *m* Komplexon-III-Lösung (3,721 g im Liter) mit Hilfe einer Mikrobürette bis Gelb. Da die Farbe gegen Ende ausblasst, muss man den Endpunkt langsam erreichen. Sobald dies anscheinend der Fall ist, erwärmt man noch etwas und gibt, wenn nötig, weiter tropfenweise Masslösung zu, bis sich die gelbe Farbe nicht mehr ändert. Mit einiger Übung lässt sich der Umschlag auf einen Tropfen (etwa 0,02 cm³) genau erfassen. Eine vollständige Bestimmung lässt sich auf diese Weise bequem in 20 min ausführen. 1 cm³ Masslösung entspricht 0,5585 mg Fe.

Die Methode wurde an defibriniertem Kaninchenblut geprüft. Die für 1 cm³ Blut benötigte Menge Masslösung lag in der Grössenordnung von 0,8 cm³; da der Umschlag auf 0,02 cm³ genau erfasst werden konnte, betrug der Titrierfehler allein 2,5 %. Man fand im Mittel aus 16 Bestimmungen 44,2 mg Fe je 100 cm³ Blut. Die mittlere quadratische Abweichung $s = \sqrt{\Sigma (x_i - \bar{x})^2/(N-1)}$ errechnete sich zu 0,93 mg %. Der Vorteil der Methode liegt darin, dass sie rasch ist und mit einfachsten Mitteln auskommt. Sie ist jedoch nur zur Bestimmung relativ grosser Eisenmengen geeignet, weshalb zur Erreichung einer befriedigenden Genauigkeit mindestens 0,5 cm³ Vollblut verarbeitet werden sollten. Für *Serum*-Eisenbestimmungen reicht sie nicht aus; dazu müssen die klassischen *kolorimetrischen* Verfahren herangezogen werden.

E. HÄBERLI

*Forschungslaboratorien der AG. vorm. B. Siegfried, Zofingen, den 22. August 1953.*

[1] G. SCHWARZENBACH, Helv. chim. Acta *28*, 828 (1945), und folgende Publikationen. – G. SCHWARZENBACH und A. WILLI, Helv. chim. Acta *34*, 528 (1951).

*Summary*

The volumetric determination of total iron in blood by titration with "Complexone III" is described. The method is simple, rapid, and accurate but not sensitive enough to permit the estimation of iron in blood-serum alone.

# Nouveaux livres - Buchbesprechungen - Recensioni - Reviews

## Methoden und Anwendungen der Massenspektroskopie

Von H. EWALD und H. HINTENBERGER

228 Seiten mit 133 Abbildungen
(Verlag Chemie, Weinheim 1952)
(Ganzleinen DM 25.60)

Die rasche Entwicklung der Massenspektroskopie im letzten Jahrzehnt machte eine zusammenfassende Darstellung der zahlreichen Einzelreferate auf diesem Gebiete sehr wünschenswert, da in dem als grundlegend angesehenen Werk ASTONS – in der zweiten erweiterten Auflage – die einschlägige Literatur nur bis zum Jahre 1941 verarbeitet ist. Es ist den Verfassern gelungen, in klarer und übersichtlicher Form sowohl die geschichtliche Entwicklung wie auch die neuen Methoden und Aufgaben der Massenspektroskopie aufzuzeigen. Um dem erhöhten Interesse, das diesem Spezialgebiet in letzter Zeit von seiten der anderen Wissenschaften, Technik und Industrie zugewendet wurde, gerecht zu werden, geht eine allgemein leichtverständliche theoretische Behandlung der ionenoptischen Eigenschaften elektrischer und magnetischer Ablenkfelder voraus. Dadurch ist auch dem Nichtfachmann die Möglichkeit gegeben, die zum Bau und Betrieb von Massenspektrographen und Massenspektrometern notwendigen Voraussetzungen in kürzester Zeit zu erfassen. Der Massenspektroskopiker wird die zahlreichen bis zum Jahre 1951 wichtigsten Literaturzitate und die Art und Auswahl des behandelten Stoffes begrüssen. Der Hauptteil des Buches ist den vielseitigen Anwendungsmöglichkeiten der Massenspektroskopie gewidmet, die zu den ursprünglichen Hauptaufgaben der Isotopenmassen- und relativen Häufigkeitsbestimmung hinzugekommen sind. Unter anderem wurden die massenspektroskopischen Methoden zur Untersuchung von Kernumwandlungen, die geologische Alters-

bestimmung, die Bestimmung des Ionisationspotentials und der Dissoziationsvorgänge sowie die routine Gasanalyse behandelt. Bemerkenswert ist die Verwendung von Massenspektrometern zur laufenden Produktionsüberwachung in der Industrie und in der Biologie und Medizin zur Stoffwechselkontrolle. Auf eine neue Art von Apparaten, den sogenannten «Time of Flight Instruments», sowie auf eine sehr erfolgversprechende Art der Ionenregistrierung mit Hilfe von Elektronenvervielfachern wird nur kurz eingegangen. Die vielen illustrierten Abbildungen bereichern das Werk, das jedem Interessenten bestens empfohlen werden kann.

M. J. Higatsberger

## Chemistry of Carbon Compounds

Edited by E. H. Rodd

Volume I B. 685 pages with 23 figures (1952)
(£5/15)
Volume II A. 487 pages (1953)
(£4/4)
(Elsevier Publishing Company, Amsterdam)

Von dem Roddschen Werk, das eine umfassende lehrbuchmässige Darstellung der ganzen organischen Chemie beabsichtigt und damit eine sehr fühlbare Lücke in der chemischen Literatur zu schliessen sich anschickt, liegen jetzt zwei weitere Teile vor. In Teil I B wird zuerst die systematische Behandlung der aliphatischen Verbindungen, nach funktionellen Gruppen und ihren Kombinationen geordnet, im wesentlichen abgeschlossen; als Autoren zeichnen wie in Teil I A wieder Johnson und Dalgliesh mit Mitarbeitern. Daran schliessen sich geschlossenere Kapitel über Monosaccharide (Jones), Polysaccharide (Percival), Proteine (Bailey) und Enzyme (Stacey) an. Ein ausführlicher Index über die Teile A und B schliesst Band I ab. – Teil II A behandelt in 9 Kapiteln aus der Feder von Raphael die synthetischen alizyklischen Verbindungen, systematisch nach Ringgrösse und Struktur (einfache und anellierte Systeme, Brückensysteme) geordnet, jedoch ohne die Terpene und Steroide, denen Teil II B gewidmet sein soll. Wenig glücklich erscheint dem Referenten ein hier eingeführtes Nomenklaturprinzip, das die räumliche Lage von *allen* Substituenten an kleinen Ringen mit *cis* oder *trans* wiedergeben möchte, ohne zuvor ein Bezugssystem zu definieren (zum Beispiel Formeln auf S. 6); es wird jedoch so vorsichtig eingeführt, dass es das Verständnis des Textes nicht beeinträchtigt. Auf diesen systematischen Teil folgt ein Kapitel über Karotinoide (Hunter) und eines über Kautschuk und künstliche Polymere (Bacon), in dem auch die technologische Seite gebührende Berücksichtigung findet.

Es ist Herausgeber und Autoren wiederum gelungen, ein gewaltiges Tatsachenmaterial auf knappem Raum nicht nur zu akkumulieren, sondern in seinen wesentlichen Zügen übersichtlich darzustellen. Trotz der Vielzahl der Autoren konnten Überschneidungen bemerkenswert gut vermieden werden; die grosse Zahl von Literaturzitaten wird das Werk zu einem wichtigen Hilfsmittel des Organikers machen. Druck und Ausstattung sind sehr ansprechend; dagegen ist es ärgerlich, dass ein Werk, das dem Benützer so rasch unentbehrlich wird wie das vorliegende, mit zahlreichen Druckfehlern behaftet ist. Es ist sehr zu wünschen, dass Herausgeber und Verlag Wege finden, um trotz erschwerender Umstände (das englisch geschriebene Werk wird in Holland verlegt und gedruckt) den folgenden Bänden auch in dieser Beziehung diejenige Sorgfalt angedeihen zu lassen, die dem hohen intellektuellen Niveau des Werkes entspricht.

H. Dahn

## Marine Geology

By Ph. H. Kuenen

568 pages, with 246 figures and 2 tables
(John Wiley & Sons, Inc. New York N.Y., 1950)
($7.50)

Die geologische Wissenschaft konnte ihr Arbeitsfeld vom Kontinent auf den Meeresboden erweitern, als 1876 das englische Forschungsschiff «Challenger» unter der wissenschaftlichen Leitung von Sir William Thomson seine vierjährige Tiefsee-Expedition erfolgreich beendete. Die moderne Technik lieferte inzwischen das Rüstzeug, dass mit jeder neuen Expedition neue, zum Teil ungeahnte Tatsachen erkannt wurden. Nach dem letzten Weltkriege sind einige sehr gute Zusammenfassungen der Probleme und Ergebnisse der submarinen Geologie erschienen. Professor Kuenen, als Ordinarius der Geologie der Universität Groningen, ist wie kein anderer berufen, ein Lehrbuch der marinen Geologie zu schreiben, weil er Geologe ist. So spricht das gut ausgestattete Werk in erster Linie den Geologen an, dann erst den Geographen und Ozeanographen.

Die meisten Gesteine sind «Kinder des Meeres». Wie und wo sind sie entstanden? Es werden deshalb in dem ersten Kapitel die ozeanographischen Umweltfaktoren behandelt, soweit sie auf die rezente Gesteinsbildung einen Einfluss ausüben, zum Beispiel die gelösten Gase und Salze im Seewasser, die Temperatur und die Farbe und Durchsichtigkeit des Wassers, die bodennahen Strömungen. Den Gezeitenströmungen wie den oberflächlichen Strömungen wird ausführlich ein eigenes Kapitel gewidmet.

Will man mit Erfolg und dann auch mit Genuss das Buch studieren, muss man die ersten hundert Seiten gründlich durchgehen, da sie das ozeanographische Material enthalten, das unentbehrlich ist, um den geologischen Teil ganz erfassen zu können

Der Ozeanboden ist morphologisch kein monotones, ebenes Gelände, wie man früher annahm, als es noch kein Echolot gab. Er hat ein Gross- und ein Kleinrelief. Wie sind die Formen entstanden? Mit welcher Sicherheit kann der Geologe die Topographie des Meeres genetisch deuten? Findet er vertraute Züge des Festlandes im Ozean wieder? Warum viele Züge ganz anders sein müssen, weist Kuenen in dem Abschnitt "Constitution of the Continents and Ocean Floors" nach. Mit neuen Erkenntnissen geht der Verfasser an das alte Problem der Permanenz und des Ursprunges der Ozeane und Kontinente heran.

Jeder Kontinent, jede grössere Insel, alle solitären Seeberge werden von einem seichten Schelf einer Kontinentalterrasse bis – 200 m etwa umgeben. Kuenen zählt viele Faktoren auf, die ihn gebildet haben. Er ist nicht nur – wie früher angenommen – von den Wellen geformt. Gerade bei diesem Kapitel kann der Geologe lange verweilen, da die meisten Gesteine von fossilen Schelfmeeren stammen. Längst ist nicht alles geklärt und gelöst, und es zählt zu den Eigenarten dieses Buches, dass darin immer wieder neue Fragen gestellt sind oder alte in neuer Sicht erscheinen.

Die Abhandlung über die marinen Sedimente selbst ist in sich ein kleines Lehrbuch und behandelt die Her-

kunft und den Transport des Materials in die See hinein und ferner alle Umweltfaktoren, welche schliesslich den Charakter des Gesteines bestimmen.

Kuenen hat sich als Teilnehmer der berühmten «Snellius»-Expedition schon früher mit den Korallenriffen beschäftigt. Hier gibt er eine vorzügliche Zusammenschau des inzwischen enorm angewachsenen Tatsachenmateriales.

Mit Anteilnahme liest man die Neuentdeckungen der submarinen Geologie: die gewaltigen submarinen Canyons, eingeschnitten in den Kontinentalabfall und die einsamen Seeberge im Pazifischen Ozean.

Geologie ist Geschichte der Erde. Dass die submarine Geologie schon Geschichte der Erde ist und in der Zukunft noch mehr wird, zeigen die letzten Seiten des Werkes, darin die eustatischen Schwankungen des Meeres im Quartär und in der Jetztzeit abgehandelt werden.

Der Referent muss gestehen, dass ihm das Buch von Kuenen viel, sehr viel gegeben hat und dass er dankbar dieses Werk für sein Kolleg und allgemeine Vorträge benutzte. Überflüssig zu sagen, dass der Form, dem Inhalt und der Bibliographie die technische Aufmachung des Buches entspricht.

M. Pfannenstiel

## Modern Trends in Physiology and Biochemistry

*Woods Hole Lectures dedicated to the Memory of* Leonor Michaelis
Edited by E. S. Guzman Barron
538 pages with 137 figures
(Academic Press Inc., Publ., New York, 1952)
($ 8.50)

Der Band enthält zwanzig Vorlesungen, die 1950 im Rahmen des Physiology Course in Woods Hole zu Ehren von Prof. L. Michaelis gehalten wurden. Geborener Deutscher, wirkte L. Michaelis lange Zeit als Biochemiker an der Johns-Hopkins-Universität und am Rockefeller-Institut; er wurde vor allem bekannt durch seine physikochemischen Untersuchungen über die Wirkungsweise der Fermente. Der ausserordentlich reiche Inhalt des Bandes, in dem erste Spezialisten über ihr Gebiet referieren, kann nur knapp angedeutet werden: E. S. G. Barron besprach fermentative Oxydation-Reduktion-Systeme, Br. Chance die Beziehungen zwischen den Enzymen und ihrem Substrat, zwei Gebiete, die besonders von Prof. Michaelis grundlegend gefördert wurden. Der Vortrag von J. Runnström behandelte die Struktur des Zytoplasmas und seine Rolle in Stoffwechsel, Wachstum und Differenzierung, der von D. Mazia Struktur und Physiologie des Zellkerns. L. V. Heilbrunn sprach über die Physiologie der Zellteilung. A. K. Parpart und R. Baliantine schilderten neue Untersuchungen über die Molekularstruktur der Erythrozyten-Zellmembran, M. H. Jacobs solche über ihre Permeabilität. Fünf Vorlesungen sind der Nervenphysiologie gewidmet: H. B. Steinbach, H. Grundfest, D. Nachmansohn und J. M. Tobias diskutierten das Kalium-Natrium-Gleichgewicht, die elektrischen Erscheinungen und die physikochemischen Veränderungen im Nerven während der Reizleitung, C. L. Prosser besprach die vergleichende Physiologie des Nervensystems. In den Vorträgen von A. Szent-Györgyi, W. F. H. M. Mommaerts und St. W. Kuffler kamen die Thermodynamik des Muskels, die Struktur seiner kontraktilen Eiweißstoffe und die Vorgänge beim Reizübertritt vom Nerven auf den Muskel zur Sprache. H. Neurath berichtete über seine Untersuchungen am Ferment Chymotrypsin, einem Protein, das wegen seiner Kristallisierbarkeit in besonders reiner Form dargestellt werden kann. G. Wald behandelte die biochemischen Veränderungen im Laufe der Evolution der Wirbellosen und Wirbeltiere. Die Wirkungsweise bestimmter chemischer Stoffe auf Proteine und Zellen wurde von O. Loewi und I. M. Klotz diskutiert, während zum Abschluss E. S. G. Barron die Reaktionswege des oxydativen Kohlenhydratstoffwechsels schilderte. In allen Vorlesungen wird das Prinzip des Physiology Course in Woods Hole deutlich: die Lebenserscheinungen im molekularen Bereich zu erklären.

H. Nüesch

# Informations - Informationen - Informazioni - Notes

## STUDIORUM PROGRESSUS

### La trame protidique des nacres[1]

Par Ch. Grégoire, Ghislaine Duchateau et M. Florkin[2], Liège

*Introduction.* Les coquilles calcaires des Mollusques sont intéressantes à des titres divers, qui relèvent de la zoologie systématique, de la biochimie et de la géochimie. Elles sont des structures complexes résultant de l'activité de diverses formations cellulaires. Dans un certain nombre d'exemples, l'un de leurs constituants est la structure qui a reçu le nom de nacre.

Il est généralement admis dans la littérature que la nacre, formant la couche interne de la coquille chez certains Pélécypodes, Gastéropodes et Céphalopodes, est constituée de lamelles très minces, alternativement organiques et calcaires (cristaux d'aragonite) disposées en assises serrées, parallèles les unes aux autres[1]. L'épaisseur des lamelles calcaires varie entre 0,6 $\mu$ et 8 $\mu$ suivant les espèces.

[1] Ces recherches feront, dans les Annales de l'Institut océanographique, l'objet d'une publication illustrée de 97 micrographies électroniques.

[2] Laboratoires de Biochimie de l'Université de Liège, Belgique.

[1] La littérature abondante concernant la question est analysée en détail dans les travaux classiques de W. von Nathusius-Königsborn (1877), R. Moynier de Villepoix (1892), O. Römer (1903), H. L. Jameson (1912), W. Biedermann (1914) et W. J. Schmidt (1924).

Les lamelles organiques sont élaborées par appositions périodiques successives du produit de sécrétion de certaines catégories de cellules de l'épithélium palléal. Ces lamelles organiques apparaissent à l'examen microscopique de la nacre polie comme des lignes de démarcation d'une finesse extrême entre les lames calcaires, relativement beaucoup plus épaisses.

La structure feuilletée de la matière organique apparaît distinctement lorsque celle-ci est libérée de sa gangue minérale par l'action d'agents décalcifiants. Elle se présente alors sous forme de membranes ou de pellicules molles superposées, translucides et iridescentes. Les données relatives à la structure de ces pellicules organiques sont peu nombreuses, et sont contradictoires. En lumière polarisante, elles seraient isotropes selon les uns[1] et présenteraient, selon d'autres[2], des phénomènes de biréfringence. Au microscope ordinaire, on n'a pu y discerner la moindre trace d'organisation, même après l'emploi de carmin boracique, de picrocarmin de Ranvier, d'hématoxyline et de teinture de cochenille, qui les colorent énergiquement[2,3].

Les seules informations que l'on ait sur la composition en acides aminés des lames organiques de la nacre sont celles qu'ont recueillies Roche, Ranson et Eyserric-Lafon[4], dont les analyses ont porté sur la matière globale des lames.

La nature très stable de la matière scléroprotéinique qui constitue les lamelles organiques de la nacre en fait un matériel convenant particulièrement à l'étude parallèle des extractions chimiques et des modifications concomitantes de la morphologie submicroscopique. Cette étude est encore favorisée par le fait que la structure feuilletée que laisse la décalcification d'une nacre ou d'une perle est clivée par l'action des ultra-sons en lamelles ou fragments de voiles d'une grande minceur, propices à l'étude au moyen du microscope électronique.

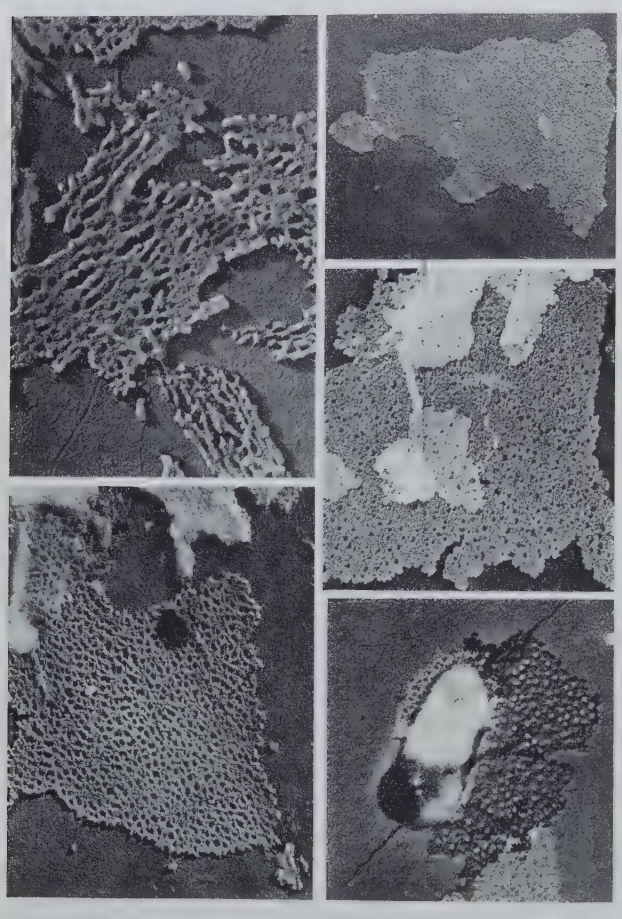


Fig. 1. *Nautilus macromphalus* Sowerly (Céphalopode). 165. 30. 12. 52 + 4 E. Ombrage à l'or-palladium. × 14500.

Fig. 2. *Astrea olivacea* Wood (Gastéropode). 133. 23. 12. 52 + 3 C. Ombrage à l'or-palladium. × 14000. – Par suite de l'absence de diaphragme dans l'objectif, la lumière du filament, transmise sans obstacle, est enregistrée au centre des clichés sous forme d'une tache ronde (en noir sur les épreuves négatives: Fig. 2, 6 et 7).

Fig. 3. *Mytilus edulis* Linné (Pélécypode). 166. 25. 3. 52 + 6 A. Ombrage au palladium. × 14000.

Fig. 4. *Pinctada* (*Pteria. Meleagrina*) *margaritifera* Linné (Pélécypode). 146. 25. 3. 52 + 9 A. Ombrage au palladium. × 14000.

Fig. 5. *Umbonium giganteum* Lesson (Gastéropode). 156. 27.11.51 + 1B. Réplique de lamelle semi-humide. Ombrage au palladium. Epreuve négative. Le moulage de la lamelle apparaît en creux, celui des pores en relief. Une bactérie (en blanc sur l'épreuve négative) a été partiellement détachée du verre avec le moulage. × 14000.

*Etude de la structure et des constituants des lamelles organiques.*

Des pellicules organiques de nacre de 25 espèces de Pélécypodes, de 15 espèces de Gastéropodes et d'une espèce de Céphalopode ont été examinées au microscope électronique (R.C.A., EMU), après ou sans immersion dans l'acide osmique (2%) et après délamination par les ultra-sons.

Les lamelles apparaissent criblées de plages claires arrondies ou allongées (cavités ou pores), qui leur donnent l'aspect de dentelles.

Le diamètre des pores, leur répartition dans les lamelles et par suite le dessin des dentelles, accusent des différences entre les trois classes de mollusques étudiés[5].

Chez *Nautilus macromphalus* (Céphalopode), les pores sont allongés, irrégulièrement ovales. Les travées de substance qui les entourent montrent des inégalités de densité ou d'épaisseur. Dans les préparations ombrées, les travées apparaissent sous forme de cordons ou de bandelettes plus ou moins aplaties, parsemées de petites élévations arrondies (Fig. 1).

Chez plusieurs espèces appartenant à différents genres de Gastéropodes, les pores, plus petits que les structures correspondantes dans les pellicules de *Nautilus*, sont généralement arrondis et le dessin des dentelles est plus régulier. Sur les préparations ombrées, les travées de substance délimitées par les pores apparaissent aussi sous forme de bandelettes occasionnellement parsemées d'élévations arrondies (Fig. 2).

Chez les Pélécypodes, la trame des dentelles est distinctement plus serrée que dans les deux classes précédentes, et les pores, très petits, sont rapprochés les uns des autres ou clairsemés (Fig. 3). Dans les pellicules de *Pinctada* (*Pteria Meleagrina*) *margaritifera*, s'observent des pores relativement larges, analogues à ceux des Gastéropodes. Ils sont toutefois moins nombreux, plus

[1] W. Biedermann, dans *Handbuch der vergleichenden Physiologie* de H. Winterstein, 3, I/1 (Verlag Gustav Fischer, Jena) 1914, 319.

[2] W. J. Schmidt, Zool. Jb. (Anat.) *45*, 1 (1924).

[3] R. Moynier de Villepoix, J. Anat. Physiol. *28*, 461 et 582 (1892).

[4] J. Roche, G. Ranson et M. Eyserric-Lafon, C. r. Soc. Biol. *145*, 1474 (1951).

[5] Notes préliminaires dans Arch. intern. Physiol. *57*, 121 (1949); *58*, 117 (1950); *58*, 483 (1951); *59*, 257 (1951); J. Physiol. *43*, 889 (1951); C. r. Congrès intern. Micr. électr., Paris, septembre 1950.

Teneur en 15 acides aminés (azote de chaque acide aminé en % d'azote total) des trois fractions de la trame protidique de la nacre d'un Pélécypode (*Pinctada margaritifera*), d'un Gastéropode (*Trochus* sp.) et d'un Céphalopode (*Nautilus macromphalus*) *

| Acides aminés | Extrait au borate (nacrïne) | | | Extrait à la soude (nacrosclérotine | | | Résidu insoluble (nacroïne) | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Pinctada* | *Trochus* | *Nautilus* | *Pinctada* | *Trochus* | *Nautilus* | *Pinctada* | *Trochus* | *Nautilus* |
| Alanine | 5,4 | 5,4 | 8,3 | 15,3 | 14,4 | 19,7 | 61,6 | 35,8 | 30,3 |
| Arginine | 13,4 | 11,4 | 8,7 | 3,9 | 5,3 | 6,0 | 2,5 | 4,7 | 3,5 |
| Acide aspartique | 16,1 | 15,5 | 23,4 | 11,2 | 4,7 | 12,4 | 1,7 | 1,3 | 1,7 |
| Acide glutamique | 4,2 | 3,3 | 4,1 | 0,09 | 1,3 | 1,2 | 0,6 | 3,8 | 3,3 |
| Glycocolle | 28,4 | 19,0 | 15,1 | 39,4 | 41,1 | 22,0 | 18,9 | 15,1 | 5,6 |
| Histidine | 0,5 | 1,2 | 0,7 | 0,02 | 0,08 | 0,05 | 0,0 | 0,1 | 0,1 |
| Isoleucine | 2,5 | 1,0 | 1,5 | 0,5 | 1,4 | 1,1 | 0,5 | 1,2 | 1,5 |
| Leucine | 4,7 | 2,7 | 3,6 | 4,7 | 1,0 | 1,5 | 4,5 | 1,8 | 1,2 |
| Lysine | 3,8 | 1,8 | 3,1 | 0,0 | 0,0 | 0,5 | 0,0 | 0,1 | 1,6 |
| Méthionine | | 0,5 | | 2,0 | 0,7 | 0,2 | 0,0 | 0,2 | 0,1 |
| Phénylalanine | 1,9 | 5,8 | 3,5 | 0,7 | 2,6 | 1,8 | 0,9 | 2,1 | 1,0 |
| Proline | 3,5 | 3,1 | 4,6 | 0,1 | 0,4 | 0,9 | 0,0 | 0,4 | 1,6 |
| Thréonine | 2,0 | 1,5 | 2,3 | 0,0 | 0,0 | 0,0 | 0,0 | 0,8 | 0,0 |
| Tyrosine | 3,6 | 2,1 | 0,6 | 0,3 | 0,0 | 0,0 | 0,1 | 0,0 | 0,0 |
| Valine | 2,2 | 2,2 | 3,6 | 1,0 | 0,7 | 1,6 | 2,1 | 1,2 | 3,0 |

* Les Nautiles sont les seuls Céphalopodes vivants dont la coquille contienne une nacre.

espacés, et les travées intermédiaires ont l'aspect de la trame serrée des Pélécypodes (Fig. 4).

Dans le matériel organique provenant de perles d'aragonite, on retrouve les lamelles perforées avec les caractères de la nacre normale correspondante.

Les différences de structure ont été appréciées quantitativement à l'aide d'un procédé élaboré par P. Bourgeois et R. Coutrez, utilisant l'élément le plus accessible aux mensurations, le pore des dentelles. Les résultats seront exposés dans un travail *in extenso*: ils corroborent les données fournies par l'examen direct des micrographies.

Les pellicules protidiques abandonnent à une solution de borate sodique, d'alcool et d'éther de pH 9,2, à 53° C, un constituant dont la teneur en 15 acides aminés, dosés par voie microbiologique, a été déterminée (voir Tableau). Après l'extraction de ce constituant, le clivage par les ultra-sons paraît s'effectuer plus rapidement que la délamination des pellicules non traitées. La structure en dentelles subsiste: on retrouve sur les micrographies les dalles ou paillettes aplaties perforées de pores. L'élargissement des pores et l'amincissement des travées ont été observés dans certaines préparations.

Un deuxième constituant peut être extrait par la soude à 5 g/100 ml agissant à 53° C pendant 5 h. Cette deuxième fraction de la trame protidique n'a pas la même composition que la première (voir Tableau), au moins en ce qui concerne les 15 acides aminés dosés: elle a notamment une teneur plus élevée en alanine et en glycocolle et une teneur moindre en arginine. Si le résidu obtenu après les deux extractions est débarrassé de la soude par lavages répétés à l'eau bidistillée, puis mis en suspension dans l'eau bidistillée, il se présente sous forme de flocons ou d'amas de filaments. L'aspect moiré, irisé, est aboli, même après action des ultra-sons. Les structures en dalles perforées de pores ont disparu. Elles sont remplacées par un matériel hétérogène, dont l'élément le plus représentatif est constitué par des réseaux ou des agrégats de fibres, fibrilles ou de filaments faiblement contrastés (Fig. 6 et 7).

Après hydrolyse, la détermination des 15 acides aminés déjà dosés dans les autres fractions ne révèle pas, dans la troisième, l'existence de tous les acides aminés habituellement présents dans les protéines. Dans la troisième fraction de la trame protidique de la nacre de *Pinctada margaritifera*, la grande huître perlière, l'azote

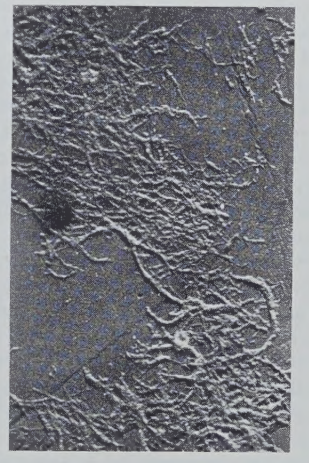


Fig. 6. *Nautilus macromphalus*. 198. 16. 6. 53 ÷ 1B. Résidu de pellicule après extraction par le borate et par la soude. Matériel non fixé, dissocié par les ultra-sons. × 14000.

de l'alanine et du glycocolle réunis constitue 80,5% (61,6 + 18,9) de l'azote total, et l'analyse n'a pas révélé

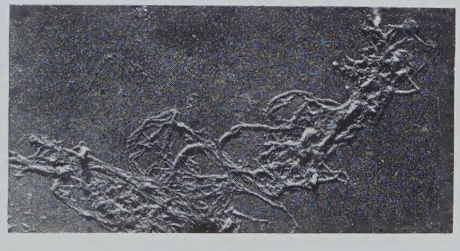


Fig. 7. *Pinctada margaritifera*. 193. 19. 12. 52 ÷ 11D. Résidu de pellicule après extraction par le borate et par la soude. Matériel non fixé, non traité par les ultra-sons. Ombrage à l'or-palladium. × 14500.

de quantités dosables, par la méthode utilisée, d'histidine, de lysine, de méthionine, de proline et de thréonine (voir Tableau).

*Discussion.* L'interprétation des structures (pores, différences dans le dessin des dentelles) doit tenir compte de plusieurs artefacts possibles, produits au cours de la préparation et de l'examen du matériel: décalcification, fixation, ultra-sons, dessiccation, bombardement par les électrons. On a cherché à contrôler ou à éviter ces artefacts.

1° Les pellicules organiques non minéralisées d'*Anodonta cygnea* possèdent des cavités ou pores semblables à ceux que l'on trouve dans les pellicules obtenues par décalcification des coquilles de la même espèce.

2° Les structures sont identiques dans le matériel fixé et non fixé. La fixation peut produire, si elle se prolonge, une rétraction modérée, mais elle n'altère pas l'aspect caractéristique du spécimen.

3° Les structures apparaissent, sans utilisation des ultra-sons, dans les lambeaux déchiquetés de voiles minces situés en bordure des fragments opaques, obtenus par broyage au mortier des pellicules organiques. L'action prolongée des ultra-sons peut provoquer en bordure des lamelles ultra-fines l'élargissement des pores et la dislocation des travées. Ces modifications sont aisément reconnaissables du fait de la présence simultanée dans les préparations de tous les stades du clivage.

4° Le contrôle des effets de la dessiccation a été réalisé à l'aide de répliques humides en formvar ou en collodion de lamelles organiques ultra-sonées et sédimentées sur verre. Dans les moulages ombrés de ces lamelles, la réplique des pores apparaît sous forme de boutons en saillie sur le plan de moulage de la surface des lamelles (Fig. 5). Il est d'ailleurs peu probable *a priori* que des artefacts de dessiccation puissent jouer un rôle important dans l'altération du présent matériel: on sait que les modifications provoquées par la dessiccation sont d'autant moins importantes que les structures contiennent moins d'eau à l'état normal. Dans les coquilles, le matériel organique enrobé dans la gangue minérale est sec ou faiblement hydraté à l'état normal.

5° Pour l'examen du présent matériel, deux sortes de canons (zero biased gun et self biased gun) ont été utilisées. L'illumination intense des spécimens a été rendue responsable d'artefacts variés, tels que vacuolisations, contractions, étirements (Richards et Anderson[1]). Ces artefacts risquent de devenir plus fréquents depuis l'emploi du self biased gun (Watson[2]). On peut les éviter en réduisant l'intensité du flux au minimum compatible avec les observations et enregistrements. Des dalles organiques ultra-sonées provenant de plusieurs espèces ont été soumises à titre de contrôle à des séries d'illuminations intenses et prolongées sans que l'on constate de vacuolisation dans les plages dépourvues de pores.

6° Un contrôle supplémentaire des sources possibles d'artefacts qui viennent d'être examinés a été réalisé à l'aide de pellicules organiques intervenant dans la constitution du calcitostracum.

On sait que malgré leur aspect nacré, la région interne de certaines coquilles est constituée, non d'aragonite, mais de calcite, et n'est pas considérée comme de la nacre véritable. La région interne de la coquille de plusieurs Pélécypodes dépourvus de nacre a été soumise aux mêmes manipulations que les pellicules de nacre. Au microscope électronique, on observe un matériel hétérogène, dont l'élément le plus représentatif est constitué par des dalles amorphes, vitreuses, parfois granuleuses, dépourvues de pores visibles, dans les présentes conditions d'observation.

Les résultats précédents semblent suggérer que les pores et les variations dans le dessin des dentelles caractérisent l'architecture normale des fines lamelles de clivage des pellicules protidiques de nacre. Les différences enregistrées dans le dessin des dentelles chez les Gastéropodes, Pélécypodes et le Céphalopode examinés possèdent la valeur d'un caractère zoologique, à cette échelle de l'organisation. Il a aussi été constaté que plusieurs espèces appartenant à un même genre présentent entre elles des ressemblances assez étroites qui distinguent leurs lamelles protidiques de celles d'espèces appartenant à d'autres genres de la même classe de mollusques.

Comme l'extraction par le borate ne modifie pas ou modifie peu l'aspect des lamelles, on peut supposer que le matériel éliminé par le borate est de faible épaisseur ou de faible densité, et qu'il est réparti dans les lamelles non traitées sous forme d'une mince couche superficielle. On peut également supposer que les structures fibrillaires, mises en évidence après action de la soude, existent à l'état normal dans les pellicules organiques, mais qu'elles y sont masquées par les substances extraites par la soude. A l'appui de cette hypothèse, on peut signaler le fait que des structures présentant certaines ressemblances avec des fibres et enrobées dans les travées des lamelles s'observent dans certaines préparations. D'autre part, sous l'action prolongée des ultra-sons, la trame de certaines lamelles se disloque en réseaux lâches, en bandelettes allongées, ondulées, parfois entrelacées en treillis et qui présentent certaines ressemblances avec les agrégats fibreux des résidus d'extraction par la soude.

La disposition des agrégats filamenteux, telle qu'elle apparaît sur les micrographies, ne correspond probablement plus à l'architecture initiale: celle-ci est modifiée par la dissociation mécanique au cours du traitement chimique. Les tensions de surface au cours de la dessiccation des suspensions sédimentées sur les films de support contribuent aussi à altérer la disposition des filaments.

*Summary*

When decalcified nacre is treated with ultrasonic waves, the organic matrix resolves itself into very thin lacelike protidic leaflets, which can be studied with the electron microscope. Controls have been made showing that the lacelike structure is not an artifact due to decalcification, fixation, treatment with ultrasonic waves, dessiccation or electronic bombardment.

The ultramicroscopic structure of the lacelike meshwork differs with the species. At this level, morphological features appear to characterize the three classes of Molluscs studied (Lamellibranchia, Gastropoda, Cephalopoda), and perhaps different genera within the same class. The extraction, by a borate buffer, of a water-soluble constituent of the protidic material does not appreciably modify the appearance of the structures studied. The extraction, by a sodium hydroxyde solution, of a second component, leaves a fibrous residue rich in alanine and glycine.

[1] A. G. Richards jr. et T. F. Anderson, J. Morphol. *71*, 135 (1942).

[2] J. H. L. Watson, J. Applied Physics *19*, 713 (1948).

Die Erforschung der Wirkstoffe des Mutterkorns beschäftigte namhafte Chemiker und Apotheker während Jahrzehnten. Einen ersten Markstein bedeutete die Entdeckung und Reindarstellung des Alkaloids Ergotamin in den Laboratorien der Sandoz A.G., wo in der Folge auch die Auffindung und Herstellung weiterer für die Medizin äusserst wertvoller Mutterkorn-Reinalkaloide gelang. Neue therapeutische Aspekte eröffnete die erstmals bei Sandoz erfolgte Hydrierung dieser Stoffe. Folgende Präparate sind das Ergebnis dieser Forschungen:

Cafergot

Dihydroergotamin-Sandoz

Gynergen

Hydergin

Methergin

Neo-Gynergen

Partergin

---

SANDOZ

S A N D O Z A. G.

---

Eine Monographie über den Stand der Mutterkorn-Forschung steht Interessenten zur Verfügung.

*Noch im Wintersemester 1953/54 erscheint:*

LOUIS F. FIESER · MARY FIESER

# *Lehrbuch der organischen Chemie*

Organic Chemistry — Second Edition
Übersetzt und bearbeitet von HANS R. HENSEL
Geleitwort von RICHARD KUHN

1954. Großoktav. 1270 Seiten.
In strapazierfähigem, säurefestem Einband DM 56.—

1. Organische Chemie auf moderner theoretischer Grundlage
2. Technische Entwicklung stets berücksichtigt
3. Ausführliches zur Pharmakologie und Chemotherapie
4. Viele chemie-geschichtliche Details
5. Deutsche Ausgabe nach neuestem Forschungsstand bearbeitet und verschiedentlich erweitert (u. a. Heterocyclen)
6. Hinweise auf zusammenfassende, leicht zugängliche Literatur aus Zeitschriften
7. Überall eingestreut Lebensdaten der Forscher
8. Straffe Auswahl durch souveräne Stoffbeherrschung
9. Flüssige, einprägsame Darstellung
10. Ein einziger handlicher Band auf Dünndruck

*Auf Wunsch ausführlicher Prospekt mit Probeseiten*

VERLAG CHEMIE · GMBH · WEINHEIM/BERGSTR.